VOLUME 51 JUNE, 1961 NUMBER 6

Phytopathology

An International Journal

Official Organ of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

UN
OF M
JUN 2 1961
SCIENCE

CONTENTS

Spread of phony disease into Georgia peach orchards — Glenn KenKnight 345

The effect of various factors on larval emergence from cysts of Heterodera glycines — D. A. Slack and M. L. Hamblen 350

Plant injury induced by ozone — A. C. Hill, M. R. Pack, M. Treshow, R. J. Downs, and L. G. Transtrum 356

Population levels of Pratylenchus penetrans as influenced by treatments affecting dry weight of Wando pea plants — James S. Dolliver 364

Tomato leaf composition in relation to bacterial spot — M. V. Nayudu and J. C. Walker 368

Host ranges and properties of some viruses from dahlia — R. A. Mildner and R. P. Scheffer 372

Relation of sugar trends and pith density to Diplodia stalk rot in dent corn — Jeweus Craig and A. L. Hooker 376

Diplodia root and stalk rot of dent corn — Jeweus Craig and A. L. Hooker 382

The development of black shank in tobacco as influenced by host nutrition — J. L. Apple 386

Effect of host exudates on chlamydospore germination of the bean root rot fungus, Fusarium solani f. phaseoli — Milton N. Schroth and William C. Snyder 389

Studies on the biology and pathogenicity of Hemicycliophora arenaria — S. D. Van Gundy and R. L. Rackham 393

Where cabbage aphids carry transmissible virus B — Gert B. Orlob and R. H. E. Bradley 397

Adaptation of Sclerotinia fructicola and Sclerotinia laxa to higher concentrations of fungicides — Rajendra K. Grover and J. Duain Moore 399

A mosaic disease of Hibbertia volubilis — Robert M. Endo 402

Phytopathological Notes

Effect of storage temperatures on the viability of lyophilized Ustilago avenae teliospores — W. T. Kondo 407

Heritable albinism in Sphacelotheca reiliana — Philip M. Halisky 407

Comparative survival of rehydrated and nonrehydrated wheat stem rust uredospores on dry leaf surfaces — Chester R. Leathers 410

Verticillium wilt resistance in peanuts (Arachis hypogaea) — T. E. Smith 411

Rhubarb root necrosis: its dissemination, effect on yield, and control by chemical soil treatments — H. H. Murakishi 412

Heat-induced susceptibility of tobacco to Tropaeolum mosaic virus — Stephen C. Y. Liu and K. M. Silberschmidt 413

The President's Column 415

News 418

Published monthly at Monumental Printing Company, Baltimore, Md.

Second class postage paid at Baltimore, Md.
Subscription, $15.00 per year

SMALL EXTRA SPRAY COST NETS BIG EXTRA POTATO YIELD

1.5% Extra Spray cost of Dithane® M-22

37% Extra Yield using Dithane M-22

Total per acre cost of producing and harvesting potatoes (using Nabam Sprays)

Usual per Acre Yield with Nabam Sprays

In growing 50 or 500 acres of potatoes, fungicide spray costs are only a fraction of the producing and harvesting costs...in the case of nabam sprays, approximately 2.5%. If DITHANE M-22 (80% maneb) is used instead, spray costs rise to about 4%, but yields may *increase as much as 37%!* In areas under heavy blight attacks, these crop increases have often been obtained in both field trials and commercial use. The small extra DITHANE M-22 cost helps produce significant extra yields. That's why it is top choice for blight control in all potato areas. Regular applications of DITHANE M-22 provide the most dependable blight protection. Write for further information on DITHANE M-22.

ROHM & HAAS
PHILADELPHIA 5, PA.

SPRAY AND SAVE WITH

DITHANE M-22

PHYTOPATHOLOGY

An International Journal, Official Organ of The American Phytopathological Society

EDITORS

R. W. FULTON, Editor-in-Chief, Department of Plant Pathology, University of Wisconsin, Madison
R. R. BAKER, Colorado State University, Fort Collins
G. W. BRUEHL, Washington State University, Pullman
E. C. CALAVAN, University of California, Riverside
J. M. DALY, University of Nebraska, Lincoln
M. W. GARDNER, University of California, Berkeley
J. G. HORSFALL, Connecticut Agricultural Experiment Station, New Haven
G. B. LUCAS, North Carolina State College, Raleigh
W. F. MAI, Cornell University, Ithaca, N. Y.
L. P. MILLER, Boyce Thompson Institute, Yonkers, N. Y.
J. H. OWEN, University of Georgia, Athens
R. F. PATTON, University of Wisconsin, Madison
W. F. ROCHOW, Cornell University, Ithaca, N. Y.
H. D. SISLER, University of Maryland, College Park
T. C. VANTERPOOL, University of Saskatchewan, Saskatoon
E. B. WILLIAMS, Purdue University, Lafayette, Ind.

BUSINESS MANAGEMENT

Business Manager, A. W. DIMOCK, R. D. 2, Ithaca, N. Y.
Advertising Manager, D. A. ROBERTS, Plant Pathology Department, University of Florida, Gainesville.

Subscriptions: Communications should be addressed to A. W. Dimock, R. D. 2, Ithaca, N. Y. **Subscription price:** $15 per year, domestic and foreign. **Single current numbers** (within 60 days of publication): $1.25. Price of back volumes and separate numbers on request. **Thirty-Year Index** (Vols. 1-30, 1911-1940) bound: $7.50. **Ten-Year Index** (Vols. 31-40, 1941-1950): paper cover, $3.50. Claims for copies lost in domestic mails must be received within 90 days from the date of issue, otherwise the replacement charge will be $1.25. Deliveries overseas are not guaranteed; foreign mailings are made at the subscriber's risk. **Separates:** no gratis copies supplied. Quotation is sent with proof. **Advertisements:** rates may be secured from the Advertising Manager. No endorsement of any statement of claims made in advertisements is assumed by this Journal or by The American Phytopathological Society.

INFORMATION FOR AUTHORS

At least one author must be a member of The American Phytopathological Society, except that any subscribing institution outside the United States or Canada may have published, by a staff member, one paper (in English) per volume. A letter of transmittal, signed by an official of the foreign institution, should accompany such manuscripts.

Subject matter should fall in the following categories:

1) Original research on some phase of plant disease.
2) Review papers developing a new concept, hypothesis, theory, or other integration of plant pathology.
3) Phytopathological Notes. These are accounts of techniques, original research, or phytopathological history of general importance, but sufficiently brief (1-4 typed pages) to need no summary. The editors will endeavor to publish notes as soon as possible after acceptance.
4) Letters-to-the-Editor. These may include concise statements of opinion on plant pathological subjects, announcements, or criticism. The editorial board reserves the right to reject letters, and to publish letters of rebuttal when appropriate.

Manuscripts should be in English, preferably on 8½ x 11 in. paper. Two copies should be submitted, typed double space throughout, including tables, lengends, and citations. Ample margins should be left on each side. Acknowledgements, author's position, acceptance date, etc., should follow authors' names, and will not appear as a footnote. Papers other than Notes may be organized conveniently under: Summary, Introduction, Review of Literature (if needed), Materials and Methods, Results, Discussion, and Literature Cited sections. Further subdivision of the Results section may be desirable. Avoid footnotes. The page number and author's name should appear in the upper right-hand corner of each manuscript page. The author's institutional address should follow the Discussion.

Tables should be numbered consecutively, and each typed on a separate page. Tables must have descriptive headings and should be understandable without reference to the text. Lower case superscript letters are to be used for footnotes to tables. Pages containing tables should follow Literature Cited, and should be numbered accordingly.

Figures (graphs, line drawings, and photographs) should add clearly to an understanding of the paper. The size and arrangement of figures should correspond to Journal page or column proportions. Combine illustrations in composite cuts when possible, and number each unit to correspond with the text figure reference, using consecutive Arabic numerals. Label each illustration in pencil on the reverse side with the figure number and author's name. Legends for figures should be typed separately on a numbered page following the tables. The allowance for illustrations for each article is one full-page cut, or its equivalent in cost in smaller cuts. Two ¼-page cuts, for example, cost nearly as much as one full-page cut. The author will be charged one-half

the excess of this cost. Color prints must be paid for entirely by the author. No charge is made for line drawings or tables unless these exceed 30 per cent of the article length.

For style details follow: Conference of Biological Editors-American Institute of Biological Sciences, Committee on Form and Style. 1960. Style manual for biological journals. Am. Inst. Biol. Sci. Washington. 86 p. See also: Price, W. C. 1954. Preparing manuscripts for Phytopathology. Phytopathology 44: 667-674, and the standing material in any number of Vol. 50 of Phytopathology. Authors are urged to have one or more colleagues read a manuscript critically before submitting it for publication. Manuscripts and editorial correspondence should be sent to the editor-in-chief. Submission implies nonsubmission elsewhere and (if accepted) no publication elsewhere in the same form without consent.

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

Founded 1908 **Incorporated 1915**

The A. P. S. Council

President, S. E. A. McCallan, Boyce Thompson Institute, Yonkers 3, N. Y.
Vice-President, C. S. Holton, Washington State University, Pullman
Secretary, George A. Zentmyer, University of California, Riverside
Treasurer, A. W. Dimock, Cornell University, Ithaca, N. Y.
Editor-in-Chief, R. W. Fulton, University of Wisconsin, Madison
President-Elect, William B. Hewitt, University of California, Davis
Retiring President, William C. Snyder, University of California, Berkeley
Councilors:
G. A. Brandes, Rohm and Haas, Philadelphia, Pa. (Northeastern)
W. J. Martin, Louisiana State University, Baton Rouge (Southern)
Arthur Kelman, North Carolina State College, Raleigh (At large)
M. B. Linn, University of Illinois, Urbana (North Central)
B. R. Houston, University of California, Davis (Pacific)
A. L. Taylor, Plant Industry Station, Beltsville, Md. (At large)
L. O. Weaver, University of Maryland, College Park (Potomac)

DIVISIONS OF THE SOCIETY

Northeastern: Pres. D. H. Marsden, Eastern St. Farmers' Exchange, West Springfield, Mass.; Vice-Pres. R. H. Daines, College of Agriculture, New Brunswick, N. J.; Secy.-Treas. W. F. Mai, Cornell University, Ithaca, N. Y.

North Central: Pres. C. M. Nagel, South Dakota State College, Brookings; Vice-Pres. A. L. Andersen, Michigan State University, East Lansing; Secy.-Treas. J. R. Wallin, Iowa State University, Ames.

Pacific: Pres. J. D. Menzies, Irrigation Experiment Station, Prosser, Wash.; Vice-Pres. W. A. Kreutzer, Shell Agricultural Laboratory, Modesto, Calif.; Secy.-Treas. C. J. Gould, Western Washington Experiment Station, Puyallup.

Potomac: Pres. A. B. Groves, Winchester Fruit Research Laboratory, Winchester, Va.; Vice-Pres. John G. Moseman, Plant Industry Station, Beltsville, Md.; Secy.-Treas. R. E. Webb, Plant Industry Station, Beltsville, Md.

Southern: Pres. A. L. Harrison, Texas Agricultural Experiment Station, Yoakum; Vice-Pres. D. E. Ellis, North Carolina State College, Raleigh; Secy.-Treas. J. P. Fulton, University of Arkansas, Fayetteville.

SUSTAINING ASSOCIATES

American Tobacco Company, Inc., Research Laboratory, 400 Petersburg Turnpike, Richmond 24, Va.

Campbell Soup Company, Camden, N. J.

The Dow Chemical Company, Midland, Mich.

E. I. du Pont de Nemours & Company, Inc., Wilmington 98, Del.

Ferry-Morse Seed Company, P.O. Box 778, Detroit 31, Mich.

H. J. Heinz Company, Pittsburgh 30, Pa.

Monsanto Chemical Company, 800 N. Lindberg Boulevard, St. Louis 66, Mo.

Rohm & Haas Company, Washington Square, Philadelphia 5, Pa.

Shell Chemical Company, Agricultural Chemicals Sales Division, 110 West 51st Street, New York 20, N. Y.

Tennessee Corporation, 900 Roosevelt Highway, College Park, Ga.

Yoder Brothers, Inc., Barberton, Ohio

SPREAD OF PHONY DISEASE INTO GEORGIA PEACH ORCHARDS

Glenn KenKnight

Plant Pathologist, CRD, ARS, U.S.D.A.
Accepted for publication October 29, 1960.

SUMMARY

Locations of phony-diseased trees were charted in young peach orchards in Peach County, Georgia. Spread into an orchard from a source at orchard edge appeared to follow the pattern: The number of diseased trees in the 1st row equals the number in the next 2 rows, in the next 4 rows, and so on in a geometric regression. Obstructions in the path of spread appeared to protect orchards to the leeward and to cause "compression of regression" in orchards to the windward. Groups of diseased trees apparently resulting from obstructions in the path of spread were regarded as analogous to sand dunes. The volume of spread from a potential source of the virus appeared to be correlated with prevailing wind direction.

Introduction.—Understanding the pattern of spread of phony disease would have obvious value for formulation of recommendations as to isolation of new planting from sources of phony virus (PV). In this study, data were obtained by charting the locations of stumps following the annual roguing of phony-diseased trees by pest control inspectors in peach orchards in Peach County, Georgia. Certain of these orchards were given alphabetic designations. Their neighboring orchards were numbered. The peach trees were planted on squares 20 by 20 ft.

Orchard I was planted in 1945; J, K, and R in 1946; G and H in 1947; L, P, and Q in 1948; E in 1949; and A, B, C, D, F, M, and N in 1950. A severe freeze in November 1950 greatly reduced the vector population in Peach County (6) and caused a reduction in incidence of phony disease beginning in 1953.

The interpretation of the data is based on the following premises:

1) *Diseased trees in a young orchard represented spread from outside the orchard.*—PV is not seed-borne, soil-borne, or transmitted by customary budding practices (1) and has never been detected in peach nursery stock. Generally, PV is confined to the genus *Prunus*, and in this study all spread was assumed to have taken place from infected peach and plum trees. PV is transmitted by several species of stem-feeding leafhoppers (2, 4, 5), of which *Homalodisca coagulata* (Say) (formerly identified as *H. triquetra* (F.)) is presumed responsible for most natural spread (3). This insect overwinters in woods (6) and cannot overwinter on land prepared for planting of peach trees.

2) *Viruliferous vectors entering an orchard were immediately infective.*—The incubation period of PV in vectors is from about 10 days to much longer (5). A vector might acquire PV from a tree and depart without transmitting PV immediately. If this happens, it affects the pattern of spread from a source within an orchard, but it affects to a much lesser degree the pattern of spread from 1 orchard to another. The source orchards in this study were large, and a large majority of viruliferous vectors probably became infective. A vector often feeds for a long time on a single plant.

3) *Diseased trees found in July of the 2nd summer represented a part of the spread occurring early in the 1st growing season.*—The period from February of the 1st year, when vectors begin leaving winter quarters (6) until July of the 2nd summer is less than the average incubation period of PV in young peach trees.

4) *Diseased trees found in July of the 3rd summer represented the remainder of the spread occurring in the 1st growing season.*—In vector (5) and scion-inoculation experiments nearly all trees successfully inoculated the 1st year showed symptoms by July of the 3rd summer. Possibly, a few diseased trees in July of the 3rd summer represented spread occurring very early in the 2nd summer, but this seems unlikely because the incubation period increases with age of test trees. Possibly, a few diseased trees in July of the 3rd year represented spread from sources within the orchard. This also seems unlikely because the feeding area of vectors on peach trees is mature wood (6) of which there is less on 1st-year than old peach trees.

The pattern in the second and third summers.—*Geometric regression.*—This pattern of initial spread of phony disease appeared: the number of diseased trees in the 1st row = the number in the next 2 rows, the next 4 rows, and so on in a geometric regression. The formula for this regression is: $y = b \log (x+1)$ where y is the slope of the line, b the regression coefficient, and x the distance measured in peachtree rows. When the formula is written $y = b \log (x+1)/\log 2$, b = the expected number of diseased trees in any regression increment and the expected number in the 1st row provided that the source is at the orchard's edge. Row numbers 1, 3, 7, 15, 31, and 63 are entered on the base line in Fig. 1-4. When 1 is added to each of these numbers and the logarithms of the sums are divided by the logarithm of 2, the quotients are 1, 2, 3, 4, 5, and 6, respectively. In Fig. 1-4 accumulative numbers of diseased trees are employed for the purpose of avoiding 0 in data.

The pattern in the ideal orchard.—Orchard E was ideal for study of initial spread. It was large, situated on level land, and had a great potential source of PV flanking the west edge. On the west edge, the southern half of orchard E was flanked by heavily phony-infested orchard 4 and the northern half by a

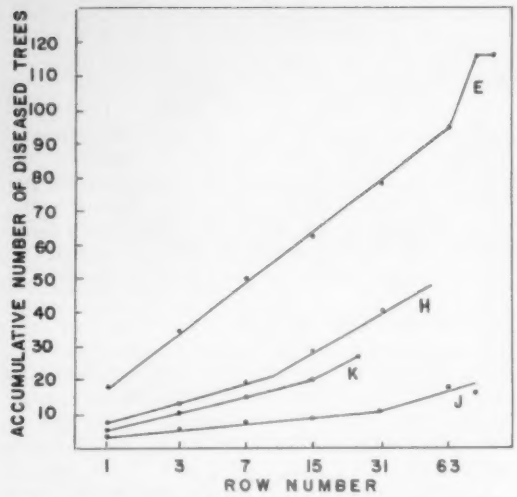


Fig. 1.—Number of phony-diseased trees in the 2nd and 3rd summers in orchards with a source of PV at an orchard edge.

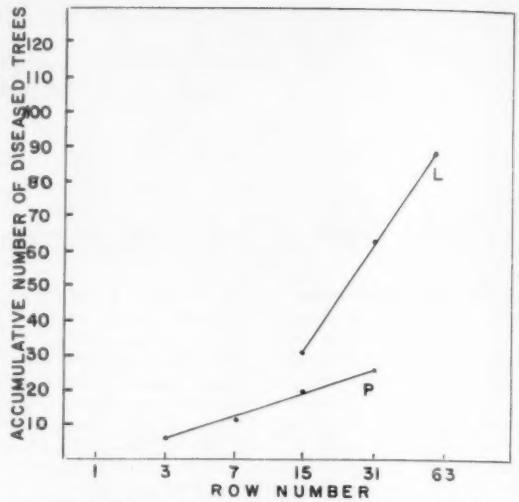


Fig. 2.—Number of phony-diseased trees in the 2nd and 3rd summers in orchards with a source of PV at a measurable distance from an orchard edge.

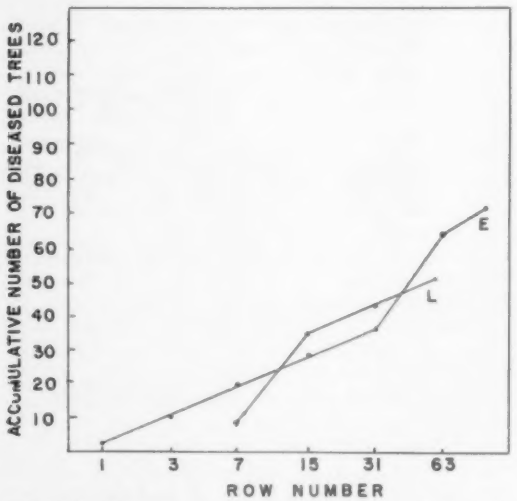


Fig. 3.—Number of phony-diseased trees in the 4th summer in orchards for which the principal source of PV was destroyed during the 1st summer.

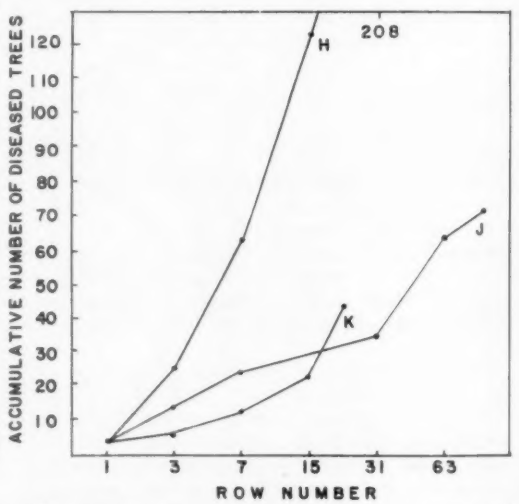


Fig. 4.—Number of phony-diseased trees in the 4th summer in orchards for which the principal source of PV was not destroyed.

corn field. There were other orchards to the south and west of orchard 4, and none in other directions. Diseased trees were rogued annually in July.

The incidence of diseased trees regressed northeastward. From the west edge, the number of diseased trees was counted among trees 1-51 in the 1st row, trees 2-52 in the 2nd row, 3-53 in the 3rd row, and so on to row 63. East of row 63, the rows were cut short at the north edge by woods and there were only 23 trees in row 93 at the edge of the woods to the east.

Within the area counted there were in the 2nd summer 7, 8, 7, 5, 4, and 2 diseased trees in the 1st, the next 2, next 4, next 8, next 16, and next 32 rows, respectively. The corresponding figures in the 3rd summer were 9, 8, 8, 9, 12, and 14; and the 2-year totals were 16, 16, 15, 14, 16, and 16 (Fig. 1).

The pattern in other orchards.—The other orchards were far from ideal for study of initial spread. Probably, in all those orchards, diseased trees represented some spread from at least 1 direction other than that measured.

In orchards J and K, diseased trees were rogued in August of the 3rd summer. The late roguing lessened the probability that all diseased trees represented spread during the 1st year.

Orchard H was flanked by old orchard 4 at the north edge and old orchard 8 at the south edge. There were wild and cultivated plum trees 300 yards across open land to the west. Spread occurred from the south and the west, but not from the north. Regression was measured in the east half of the orchard in rows of 25 trees northward 45 rows to the edge of orchard 4.

Orchard K was flanked on the north edge by orchard 11 and on the west edge by orchard 12. Spread occurred both from north and west. Regression was measured in the southern half of the orchard from the west edge in rows of 23 trees eastward 23 rows to a paved road.

Orchard J was flanked on the south edge by old orchard 11 and orchard 12 was situated 300 yards across open land to the southwest. Spread occurred both from the south and the southwest. Regression was measured from the south edge in rows of 21 trees northward for 80 rows to a pecan orchard at the north edge.

The southern half of orchard L was situated 56 yards east of old orchard 14, and the northern half was flanked on the north and west edges by woods, with old orchard 13 situated at the northwest corner. Spread occurred both from the west and the northwest. Regression was measured in the southern 25% of the orchard from the edge of orchard 14 in rows of 15 trees eastward 57 rows to a field at the east edge.

Orchard P was situated at the south edge of an unpaved road. Old orchard 22 flanked the north edge of the road. Regression was measured from the edge of orchard 22 in rows of 13 trees southward to row 31. Beyond row 31 there were a few wild plums at the west edge of the orchard.

The fit of data to geometric regression is shown in Fig. 1 for orchards with a source of PV at orchard edge and in Fig. 2 for orchards with a source at a measurable distance from orchard edge. In all instances, the fit was good for a least a short distance. The departures from geometric regression at greater distances from source were presumed due to spread from a direction or directions other than the 1 measured.

In orchard H there were 6, 6, and 5 diseased trees in the 1st, next 2, and next 4 rows. The corresponding figures for orchard J were 1, 3, and 2; for orchard K, 6, 4, and 4 (Fig. 1). In orchard P, the 1st 2 rows were regarded as missing; there were 6, 9, and 7 diseased trees in rows 4–7, 8–15, and 16–31. In orchard L, the 1st 7 rows were regarded as missing; there were 31 and 31 diseased trees in rows 8–15, and 16–31.

Alteration of pattern by obstructions in the path of spread.— 1) *Buildings.*—It is well-known that phony-diseased trees occur in groups near buildings. An example of this phenomenon was observed in orchard E. A building, 20 x 80 ft., was situated within orchard E near the northeast corner of source orchard 4. In both the 2nd and 3rd summers, there were few diseased trees near this building and there were groups of diseased trees to the north and northeast. These groups were regarded as analogous to sand dunes.

2) *Woods.*—In orchard E a band of high incidence occurred back 75–150 yards from tall woods (maximum height of trees 80 ft.) at the east edge. This band extended from the south edge northward to within 100 yards of woods at the north edge. In orchard M, groups of diseased trees occurred back 75–150 yards from woods at the north edge. It was presumed that these groups of diseased trees represented infections by vectors that might have traveled much farther in the absence of woods, and that the net result was "compression of regression." The vectors usually make low flights (6). Thus an old peach orchard should be more or less effective as a wind-break.

3) *Hills.*—Three orchards, A, B, and C, were on rolling land, and in these orchards the distribution of diseased trees appeared to be related to the lay of land. There were few diseased trees on downward slopes and groups of diseased trees on hillsides facing the presumed source.

Alteration of the pattern at orchard edges.—In 3 instances, there was an excessive number of diseased trees near an orchard edge.

The south edge of orchard E was flanked by a small-grass pasture, and the orchard in the 1st year was interplanted with cotton. The pasture, about 80 A, was flanked on the west by old peach orchards and on the east by woods. In the 2nd and 3rd summers, there were 117 diseased trees in the southwestern corner of the orchard, 60 x 60 rows. Of these 117 trees, 27 were in the southernmost 6 rows. Likely some vectors crossed the pasture and entered orchard E at the south edge; however, there was only a slight regression trend from the south edge northward. The high incidence was confined to 6 rows with the number of diseased trees in each varying only from 5 to 3.

A pattern similar to that along the south edge of orchard E occurred along a part of the south edge of orchard C, which was also interplanted with cotton and flanked by grassland.

The east edge of orchard H, which was not interplanted with another crop, was flanked by the same pasture that flanked the south edge of orchard E. In orchard H, there was no unusually high incidence along the edge of the pasture.

The suggested explanation is based on the feeding habits of the vectors. The principal vector does not feed on small grasses, whereas cotton is a favored and peach an acceptable food plant. Vectors leaving the orchards encountered non-food plants and turned back, subjecting the neighboring peach trees to double jeopardy of infection.

The pattern in the fourth summer.—The pattern of diseased trees in the 4th summer was always different from the pattern in the 3rd summer. The diseased trees were presumed to represent spread that occurred during the 2nd summer. The difference in the pattern was interpreted to mean a change in source of virus. Nearly all orchards had potential sources of PV in more than 1 direction. A shift in prevailing wind direction at a critical time during the 2nd summer or a shift in the relative numbers of vectors within source orchards would result in a change in the pattern of diseased trees in the young orchard. Spread from sources within the orchard also would change the pattern.

Trees in old orchards 4 and 14, the principal sources for orchards E and L, respectively, were uprooted in the 1st summer, the land was tilled, and the vector population forced to migrate. In orchards E and L, incidence of phony disease was high in the 3rd summer and decreased in the 4th summer (Fig. 3). Trees in the principal source orchards for orchards H, J, and K were present during the 2nd summer, and phony incidence in orchards H, J, and K increased in the 4th summer (Fig. 4). This observation led to the conclusion that many diseased trees in the 4th summer represented spread from outside the orchard. This seemed logical since the target area, mature wood, increased greatly from the 1st to the 2nd summer; and the acquisition area, mature wood, was much greater on diseased trees in the old orchards than on diseased trees in the young orchards.

In orchard E, the 4th-year incidence of diseased trees regressed north northeastward and a zone of high incidence occurred back from woods near the north edge. In this zone were 39 diseased trees in an area having 1 diseased tree in the 2nd summer and 2 in the 3rd summer. The 4th-year pattern suggested that many of the diseased trees represented spread from orchard H, 200 yards to the southwest, or from more distant orchards.

The effect of orientation of source.—Spread of phony disease from sources to the southwest appeared greatest; from the west, next; from the south, usually greater than from the north; and from the east, least.

Some old orchards were potential sources for 2 or more young orchards. Orchard 8 had about 2,700 trees planted about 1939. In 1947, orchard Q was planted at the west edge, orchard G at the northwest corner, and orchard H at the north edge of orchard 8. Spread northward into orchard H was great (Fig. 1). Little or no spread appeared westward into orchard Q; moderate infestation in that orchard apparently resulted from spread from wild plums at the north edge and 200 yards to the west. Little or no spread appeared from orchard 8 into orchard G; heavy spread into the latter apparently came from wild and cultivated plums to the west and southwest.

Orchard 4 had about 4,000 trees planted about 1938. In 1946, orchard H was planted at the south edge, orchard G at the southwest corner, and orchard R at the west edge; in 1949, orchard E was planted at the east edge of orchard 4. Spread eastward and northeastward into orchard E was great (Fig. 1), and southward into orchard H doubtful. Little or no spread appeared southwestward into orchard G and westward into orchard R. The few diseased trees in orchard R apparently resulted from spread from wild plums 400 yards to the west.

Orchard 11 consisted of about 1,000 trees planted about 1938. In 1946 orchard J was planted at the north edge and orchard K at the south edge. The first summer the northward spread into orchard J (Fig. 1) appeared to be greater than the southward spread into orchard K. However, in the fourth summer diseased trees in orchard K showed a regression trend from north to south, suggesting heavy spread from the north in the second summer.

Orchard A was flanked on the north and south edges by wild plums. Spread from the south appeared to be 3 times that from the north. Orchard I was flanked on the northwest and east edges by wild plums. Spread from the northwest appeared to be great and from the east doubtful. Orchard S was flanked on the west and southwest edges by old orchards. In the fourth summer the stand of trees was reduced to 30 percent by roguing of diseased trees.

Exposure of source.—A potential source of PV seemed ineffective if separated from a young orchard by a substantial windbreak. Protection by windbreaks was noted in parts of orchards E, I, L, and M. Also, a windbreak to the windward of a potential source appeared to reduce spread as noted in orchards M, N, and a part of I.

Discussion.—Wolfenbarger (9, 10) reviewed the literature on dispersion of small organisms. In his review, he included vertical dispersion as well as lateral dispersion, dispersion from a point or a line as well as from a large area, and dispersion of released insects as well as continuous dispersion. He fitted nearly all data to the formula:

$$y = a + b(\log x) \qquad (1)$$

or to the formula:

$$y = a + b(\log x) \div c/x \qquad (2)$$

where x is a unit of distance and a, b, and c are constants.

Apparently, few plant pathologists presented data representing primary spread of a causal organism of a plant disease exclusive of secondary spread. With reference to plant viruses, perhaps none did. Wilson and Baker (7, 8) presented data on primary spread of *Sclerotinia laxa* and of *Puccinia coronata*. They fitted the data to a formula containing only 1 constant:

$$y = 100\ (1 + a)^2 \div (x + a)^2 \qquad (3)$$

The dispersion of *S. laxa* represented invasion of an orchard from an infested orchard for a distance of 4 apricot tree rows. The spread of *P. coronata* represented dispersion from a line.

Almost any set of dispersion data may be fitted to formula 1 or 2. Many sets of data, cited by Wolfen-

barger, that represent lateral dispersion from a point or a line may be fitted to formula 3.

In this study, phony diseased trees represented invasion of young peach orchards by viruliferous vectors. Phony diseased trees in orchard E, for example, presumably represented dispersion of viruliferous vectors from each of the 50 rows of trees in neighboring infested orchard 4. The resulting data gave a good fit to the formula

$$y = b \log (x + 1) \qquad (4)$$

Possibly some modification of formula 3 would be more appropriate.

In an area where phony disease infestation is high and at a time when the vector population is also high, it may not be possible to establish a new peach orchard at sufficient distance from sources of PV to exclude the disease. In this study isolation reduced losses. Orchard D was situated 1,000 yards northeast and orchard F 660 yards east of the closest great sources of PV; in these orchards in the 3rd year, there were 3 and 2 diseased trees, respectively. In the area in orchard E illustrated in Fig. 1, 11 rows included 561 trees and was a distance of about 100 yards measuring northeastward. In the 1st 11 rows, there were 57 diseased trees; then 12, 9, 5, 6, and 6, respectively, in the next 5 blocks of 11 rows each. In this instance, 100 yards isolation greatly reduced incidence, 200 and 300 yards isolation reduced incidence further, and 400-500 yards isolation was no better than 300 yards. The ground cover was young peach trees interplanted with cotton. Perhaps, a ground cover of plants less attractive to the vectors would be less effective in trapping the vectors. The isolation was in the direction of the prevailing wind.

The importance of a phony-infested orchard as a potential source of the virus for infection of younger plantings appeared affected by orientation of the orchards and exposure to wind. There appeared little need for isolation of a young orchard from an old orchard to the east or from an old orchard separated from the young orchard by a substantial windbreak. A windbreak on the leeward side of a young orchard, however, reduced the value of isolation.

Presumably, the importance of a phony-infested orchard as a potential source of the virus for infections of younger plantings in the path of spread was more or less proportional to the number of affected trees not yet rogued plus the number of trees in incubation-stage times the number of vectors in the source orchard. To a large extent, the vector population is determined by cultural practices such as cultivation, use of organic insecticides, and use of speed sprayers that spray the orchard floor as well as the trees because the vectors breed on weeds and certain crop plants (6).

In some instances, a relatively few wild plum trees near the orchard edge were a greater source of inoculum than many affected peach trees and than a great many peach trees in incubation stage in a neighboring orchard. The common wild plum, *Prunus angustifolia* Marsh., of Peach County, propagates by underground stolons, and great numbers of trees in a thicket may be on a connected root system. PV moves through the root system, so the virus from 1 successful inoculation made by vectors may invade many trees in a thicket. As a consequence, phony disease spreads more rapidly in this plum than in peach. Not only was phony incidence higher in wild plums than in peach, but the wild plums received no insecticidal spray applications, whereas peach trees received several to many each season.

In young peach orchards, all phony-diseased trees in the 2nd year, all or nearly all of the phony-diseased trees in the 3rd year, and the majority of those in the 4th year apparently resulted from spread from outside the orchard. All spread during the 1st year and much of the spread during the 2nd year took place before an axe was laid to the 1st phony-diseased tree.

LITERATURE CITED

1. Hutchins, L. M. 1933. Identification and control of the phony disease of the peach. Georgia Off. State Entomol. Bull. 78. 55 p.
2. Turner, W. F. 1949. Insect vectors of phony peach disease. Science 109:87-88.
3. Turner, W. F. 1952. The role of insect surveys in virus-vector research. Plant Disease Reptr. Suppl. 211:47-50.
4. Turner, W. F., and H. N. Pollard. 1955. Additional leafhopper vectors of phony peach. J. Econ. Entomol. 48:771-772.
5. Turner, W. F., and H. N. Pollard. 1959. Insect transmission of phony peach disease. U. S. Dept. Agr. Tech. Bull. 1193. 27 p.
6. Turner, W. F., and H. N. Pollard. 1959. Life histories and behavior of 5 insect vectors of phony peach disease. U. S. Dept. Agr. Tech. Bull. 1188. 28 p.
7. Wilson, E. E., and G. A. Baker. 1946. Some aspects of the aerial dissemination of spores, with special reference to conidia of Sclerotinia laxa. J. Agr. Research 72:301-327.
8. Wilson, E. E., and G. A. Baker. 1946. Some features of the spread of plant diseases by air-borne and insect-borne inoculum. Phytopathology 36:418-432.
9. Wolfenbarger, D. O. 1946. Dispersion of small organisms, distance dispersion rates of bacteria, spores, seeds, pollen and insects; incidence rates of diseases and injuries. Am. Midl. Naturalist 35:1-152.
10. Wolfenbarger, D. O. 1959. Dispersion of small organisms. Incidence of viruses and pollen; dispersion of fungus spores and insects. Lloydia 22:1-106.

THE EFFECT OF VARIOUS FACTORS ON LARVAL EMERGENCE FROM CYSTS OF HETERODERA GLYCINES

D. A. Slack and M. L. Hamblen

Professor and Research Assistant, respectively, Department of Plant Pathology, University of Arkansas, Fayetteville.

Published with the approval of the Director of the Agricultural Experiment Station, University of Arkansas. Accepted for publication November 7, 1960.

SUMMARY

The effect of various factors on the emergence of larvae from *Heterodera glycines* cysts was studied under controlled laboratory conditions. Leachate or diffusate from soybean roots failed to stimulate larval emergence. A significantly greater number of larvae emerged from plump, light-brown cysts than from shrunken, dark-brown cysts or from white or yellow females. Larval emergence from cysts collected on a 40-mesh sieve was not significantly different from smaller cysts that passed through a 40-mesh sieve and were collected on a 60-mesh sieve. Larvae emerged from cysts incubated at constant temperatures maintained at increments of 4° between 16° and 36°C, but failed to emerge at higher or lower temperatures. Larval emergence was optimum at 24°C. After incubation of cysts at 40°C for 10 days, larvae failed to emerge from cysts when transferred to 24°C. Alternating the incubation temperature between 24 and 16°C reduced larval emergence. Subjecting cysts to –24°C reduced larval emergence from cysts when transferred to 24°C, but failed to eliminate emergence after exposure for 18 months. Emerged larvae were killed by freezing and by exposure to 40°C. Larval collections from Baermann funnels were increased by rupturing cysts. Larvae continued to emerge from cysts after incubation at 24°C for 12 weeks, but 87% emerged during the first 6 weeks. Light failed to affect larval emergence from cysts. Lack of aeration restricted larval emergence from cysts. Larval emergence was greatly reduced by mild desiccation of cysts.

Introduction.—Knowledge is meager on the factors that influence the development and behavior of the soybean-cyst nematode *Heterodera glycines* Ichinohe, 1952. Since second-stage larvae that have emerged from the cysts are the infectious stage, it is desirable to elucidate the conditions that regulate this population. Ichinohe (16) and Skotland (22) reported that root leachates failed to stimulate emergence of larvae from cysts. According to Skotland (22) and Epps (8), desiccation of cysts markedly reduced the "viable contents" of cysts. Ichinohe (16) reported that cysts exposed 7 months to –40°C still contained viable contents and that 10°C was the "threshold" temperature for nematode development. Winstead and Skotland (29) reported that a water temperature of 48–49°C was lethal to cyst contents.

This paper presents results of investigations on the influence of various factors on the emergence of larvae from *H. glycines* cysts. Preliminary reports have been presented (13, 23).

Materials and methods.—The original source of *H. glycines* was infested soil collected from fields in Mississippi County, Arkansas. Cysts were extracted from the naturally infested soil and used to infest methylbromide-fumigated soil in flats. Soybeans were grown in the artificially infested soil in the greenhouse to increase the cyst population used in the various experiments. When cysts were required in the laboratory, they were extracted by agitating an aliquot of heavily infested soil in water, pouring the water and suspended cysts through a 25-mesh sieve, and collecting the cysts on a 60-mesh sieve. The cysts were washed from the sieves, collected in Petri dishes, individually selected under the stereoscopic microscope, and separated into test lots at random. After preliminary investigations, a standard method was adopted for conducting the tests on larval emergence from *H. glycines* cysts. The procedures involved modifications of the hatching tests with cysts of *H. rostochiensis* Wollenweber as reported by Fenwick and Widdowson (11). In the majority of the tests, cysts were incubated in Syracuse watch glasses containing a small volume of distilled water. The incubation temperature was 24°C except when temperature was the variable being studied. The number of selected cysts used for each replicate sample was 50, and each treatment was replicated 4 times. Tests that composed a single experiment were repeated 3 times and used different lots of cysts. Since each lot of cysts denoted a different potential for larval emergence, an index of emerged larvae was derived to allow a direct comparison of data among the tests and to present the results from the various experiments more clearly. The emerged larvae index for each test was obtained by use of the formula—

$$\text{emerged larvae index} = \frac{\text{larvae emerged from each replicate}}{\text{larvae emerged from all replicates}} \times 1000.$$

The fractional number was multiplied by 1000 to retain the identity of small numbers The data from the 3 tests were subjected to a combined analysis of variance. In summarizing the results of a particular experiment, the replicate indices were averaged and illustrated graphically.

Results.—*Soybean-root leachate.*—A study was made to ascertain the influence of leachates from soybean roots and diffusate from root segments on the emergence of larvae from cysts. Soybean root leachate was collected in a manner similar to that reported by Fenwick and Widdowson (11). Root diffusate was supplied by placing segments of live soybean roots in distilled water with the cysts. Distilled water was employed as a control. The cysts used were ex-

tracted singly and briefly washed in distilled water before being placed in the various hatching media. The randomized and replicated lots of cysts were incubated at 24°C. Emerged larvae were counted and removed at weekly intervals and the hatching medium renewed. Larval emergence was not statistically different, regardless of the medium in which the cysts were incubated.

Ichinohe (16) and Skotland (22) reported that root diffusates of several host and non-host plants failed to stimulate emergence of larvae from *H. glycines* cysts. Root diffusates from host plants were reported to stimulate larval emergence from cysts of all species of *Heterodera* except *H. major* (Schmidt) Franklin and *H. gottingiana* Liebscher (15, 28).

Maturity and condition.—Observations of the populations recovered from field soils indicated that the degree of maturity of females and the maturity, condition, and size of cysts might influence potential larval populations in the soil. Microscopic examinations revealed that: 1) white females contained relatively few eggs, most of which lacked embryonic development; 2) yellow females contained a moderate number of eggs in various stages of embryonic development; 3) plump, light-brown cysts contained many eggs with larvae inside; 4) brown, shrunken cysts contained a moderate number of eggs with larvae inside; 5) brown, shrunken cysts that floated on water contained some egg shells and relatively few eggs, all with larvae inside. The association of color with maturity of the female and development of the cyst agreed with observations reported by Ichinohe (16).

In laboratory studies randomized and replicated tests were used to determine the effect of the various female and cyst conditions on larval emergence. As shown in Fig. 1-A, a significantly greater number of larvae emerged from cysts that were light-brown and plump than from cysts in other categories. The number of larvae that emerged from yellow females was significantly higher than that from white females or the shrunken cysts, although only about one-third that from light-brown and plump cysts. During incubation, the color of the females changed toward that of the next maturity group. However, the change was not rapid and the original color category was retained at the conclusion of the tests.

The influence of cyst size on larval emergence was studied. Light-brown and plump cysts collected on a 40-mesh sieve were compared to similar cysts that had passed through a 40-mesh sieve and were retained by a 60-mesh sieve. There was no significant difference in emergence.

According to Duggan (5), six-week-old white "cysts" of *H. schachtii* Schmidt failed to provide any reproductive potential when mangolds were planted in inoculated tubes of soil. Jones (17) and Ellenby (6) reported that *H. schachtii* cysts required a period of maturation before newly produced eggs were stimulated to hatch. However, larvae were reported by Jones (17) to be liberated by mature white cysts.

Constant temperature.—To determine the effect of constant temperature on larval emergence from cysts, an experiment was conducted with incubators maintained at 4° increments from 0 to 40°C. Emerged larvae were removed and counted at weekly intervals until emergence was negligible. After removal of the larvae, distilled water adjusted to the temperature of incubation was added to renew the hatching medium.

Larvae failed to emerge from cysts incubated below 16° or above 36°C. Larval emergence increased with each 4° increase in temperature from 16° to 24°C, and then decreased with each increase in temperature from 24° to 40°C. As summarized in Fig. 1-B, the number of larvae that emerged was significantly higher at 24° than at other incubation temperatures; emergence of larvae at 20°, 28°, and 32°C was similar, but significantly higher than that recorded at 16 or 36°C.

After 5 weeks, *H. glycines* cysts maintained at temperatures at which larvae failed to emerge, were transferred to 24°C. Following transfer to the optimum temperature, larvae emerged from cysts incubated at 12°C or below; total emergence, however, was considerably below that from cysts incubated originally at 24°C. Larvae failed to emerge from cysts incubated at 40°C. Incubation of cysts between 0°C and 12°C for one week or less failed to reduce larval emergence when placed at 24°C. Emerged larvae were killed almost immediately wherever ice crystals formed in the medium, but persisted for long periods at cool temperatures above the freezing point. Further tests on cysts incubated at 40°C showed that larval emergence was greatly reduced after one week and that larvae failed to emerge after 10 days when the cysts were transferred to 24°C. Emerged larvae were killed rapidly when subjected to 40°C.

In studies on the effect of temperature on emergence of larvae from *H. rostochiensis* cysts, Fenwick (10) reported increased emergence from 15 to 25°C and inhibited emergence at 30°C. In similiar studies, Lownsbery (20) reported increased larval emergence from *H. rostochiensis* cysts as the temperature increased from 15°C to an optimum between 21° and 24°C; larvae emerged at 30° but not at 33°C. Feldmesser and Fassuliotis (9) reported that some larvae emerged from *H. rostochiensis* cysts incubated at 40°F (4°C). In 1922, Baunacke (1) reported optimum larval emergence from *H. schachtii* cysts at 25°C, and reduced emergence with increased or lowered temperatures. Wallace (25) substantiated the findings of Baunacke and established that larval emergence from *H. schachtii* cysts was almost nil at 10° and 35°C.

Alternating temperature.—The effect of fluctuating temperatures on emergence of larvae from cysts was studied by subjecting cyst lots to the following temperature conditions: 1) A constant temperature of 24°C; 2) alternating temperatures of 24°C for 20 hours and 16°C for 8 hours; 3) alternating temperatures of 24°C for 16 hours and 16°C for 8 hours; 4) alternating temperatures of 24°C for 8 hours and 16°C

for 16 hours; 5) alternating temperatures of 24°C for 4 hours and 16°C for 20 hours; 6) a constant temperature of 16°C. Cysts incubated for 16 or more hours at 16° or 24°C were kept at those temperatures for 2 days each week since it was inconvenient to make the transfer during the weekends. Larvae were collected and counted at weekly intervals for 6 weeks.

As shown in Fig. 1-C, larval emergence from cysts was directly proportional to the time the cysts were incubated at 24°C. As the test progressed, however, the comparative rate of larval emergence decreased more rapidly for cysts incubated at 24°C. With *H. rostochiensis* and *H. schachtii*, respectively, Bishop (2) and Wallace (25) reported that a fluctuation of

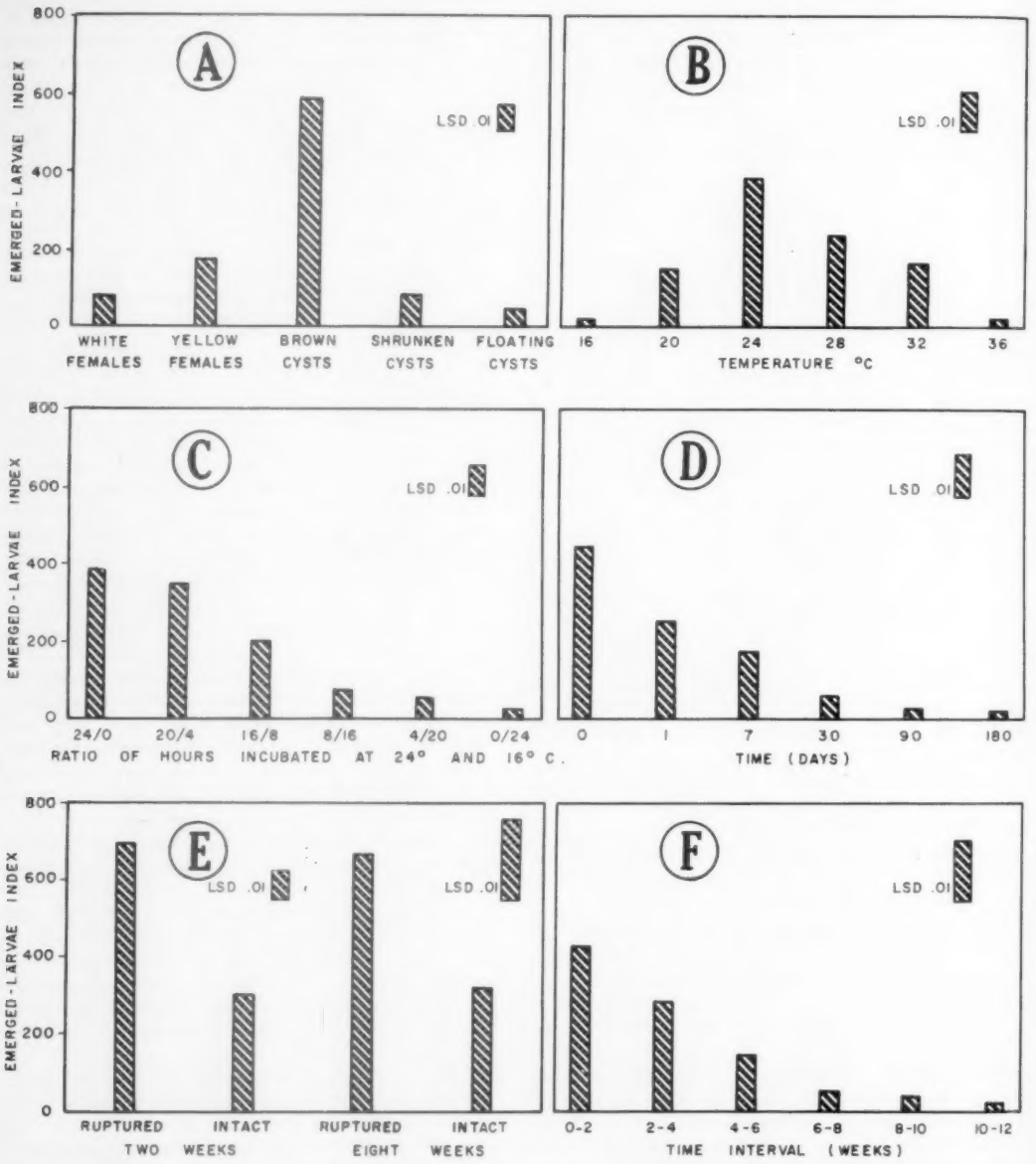


Fig. 1.—Larval emergence from *H. glycines* cysts: **A)** of different maturity and condition; **B)** incubated at a constant temperature; **C)** incubated at fluctuating temperatures; **D)** subjected to –24°C for various periods before incubation at 24°C; **E)** collection of larvae from ruptured and intact cysts placed on Baermann funnels; **F)** rate of larval emergence from cysts incubated at 24°C.

temperature increased the rate of larval emergence from cysts.

Sub-freezing temperature.—In the field during winter months, cysts are frequently subjected to temperatures below freezing. Tests were initiated in the laboratory to determine the effect of subjecting cysts to extreme cold for various periods. Syracuse watch glasses, each containing a lot of 50 cysts in a small volume of water, were placed at −24°C for different periods. Four lots of cysts were removed at specified intervals and incubated at 24°C for 8 weeks. Larvae were collected and counted at weekly intervals.

Larvae emerged from frozen cysts following transfer to 24°C. Emergence of larvae from cysts was reduced in direct proportion to the length of time the cysts remained frozen. The rate of larval emergence was relatively low during the first week and increased to a high during the second week. In some instances, the emergence of larvae remained at a low level, most frequently associated with prolonged freezing of cysts. Exposure of cysts to −24°C for as long as 18 months failed to eliminate larval emergence when the cysts were placed at a favorable temperature for emergence. Results are in Fig. 1-D.

In Japan, Ichinohe (16) observed that *H. glycines* cysts exposed to −40°C for 7 months contained viable contents. Baunacke (1) reported that emergence of larvae was not diminished when cysts of *H. schachtii* were kept in an ice block at −9°C for 6 days. Working with *H. rostochiensis*, Boyd (3) reported that emergence of larvae from dry cysts, cysts placed in water for only a short period, and cysts pre-soaked in water for 6 days, was reduced by overnight exposure to natural frost but not to exposure at −5°C in a refrigerator for 24 hours or 7 days.

Rupture of cyst.—In the genus *Heterodera*, the cyst wall not only serves as a protective body for enclosed eggs and larvae but also constitutes a barrier to emergence of the larvae. The extent to which the cyst wall restricts emergence was studied. Eight lots of 100 cysts each were selected. Four of the lots were placed on Baermann funnels and the remainder blended in a Waring Blendor for 5 minutes in sufficient water to cover the blades. This treatment ruptured about 90% of the cysts, with little apparent damage to larvae or eggs. The resultant suspension was separated into 4 equal aliquots and placed on Baermann funnels. Larvae were collected from the Baermann funnels and counted at weekly intervals. The test was repeated 3 times.

Rupture of cysts in the Waring Blendor significantly increased the collection of larvae from the Baermann funnel. Almost 90% of the larvae from ruptured cysts were collected during the first 2 weeks, and relatively few larvae were collected after one month. From non-ruptured cysts, the percentage of larvae collected during the first 2 weeks was about equal to that collected subsequently, and some larvae were collected over a 3-month period. The relative number of larvae collected after 2 and 8 weeks are presented in Fig. 1-E.

Ellenby (7) reported that larval hatch was much greater from *H. rostochiensis* cysts cut in half or from free eggs than from intact cysts.

Rate of emergence.—An experiment was conducted to determine rate of larval emergence. Tests were conducted on randomized and replicated cyst lots incubated in Syracuse watch glasses at 24°C. Emerged larvae were collected and counted at weekly intervals until emergence was negligible.

In general, the rate of emergence was inversely proportional to the length of time the cysts were incubated. Forty-three per cent of the total larvae emerged from cysts during the first 2 weeks of incubation. After one month 72% of the total larvae had emerged, and the percentage increased to 87 after 6 weeks. After 12 weeks larval emergence from cysts was practically nil. The relationship of larval emergence per unit of time is summarized in Fig. 1-F.

A similar sigmoid hatching curve was reported by Winslow (28) for various species of *Heterodera;* the rate of emergence, however, was faster.

Light.—The presence or absence of light exerts an influence upon many organisms; thus, an experiment was conducted to determine the possible influence of light on the emergence of larvae from cysts. Randomized and replicated cyst lots in Syracuse watch glasses were separated into 2 groups. One group was exposed to constant light from two 4-watt fluorescent light tubes 6 in. away. The second group was wrapped in opaque paper to exclude light and placed adjacent to the first group. The temperature was maintained at 24°C and similar in the 2 groups. Emerged larvae were removed and counted after 2 weeks.

Statistical analysis of the data indicated that the presence or absence of light did not influence emergence of larvae from the cysts. Lownsbery (19) and Fenwick (10) reported that light inhibited larval emigration from cysts of *H. rostochiensis*. According to Winslow (28), light did not influence larval emergence from *H. major* cysts.

Aeration.—A study was made of the influence of aeration on the emergence of larvae from cysts. Vessels containing different depths of water were used for rough regulation of the availability of atmospheric gases to the cysts. Replicate lots of cysts were incubated for 4 weeks in: a sufficient volume of water (5 ml) in Syracuse watch glasses to cover the cysts; water maintained ½ in. deep in a glass tube; a glass tube filled with water to an 8-in. depth and sealed.

As shown in Fig. 2-A, a significantly greater number of larvae emerged from cysts incubated in the slight water covering than from cysts placed in water maintained at greater depths. Although not included in Fig. 2-A, similar numbers of larvae were recorded from cysts incubated in water in Syracuse watch glasses and on Scotties tissue arranged in covered Petri dishes to supply water as a wick, a technique somewhat similar to that reported by Den Ouden (4) and Shepherd (21). Lack of oxygen was reported by Wallace (26) and Triffitt (24) to inhibit emer-

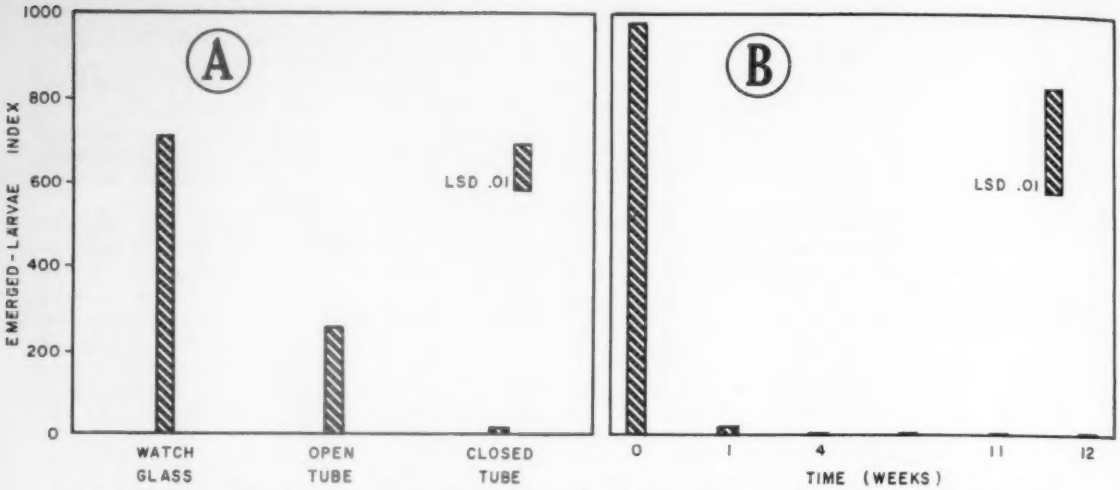


Fig. 2.—Larval emergence from *H. glycines* cysts: **A)** incubated under various conditions of aeration; **B)** air-dried in the laboratory.

gence of larvae from cysts of *H. schachtii* and *H. rostochiensis*, respectively. Skotland (22) found no difference in the number of larvae collected from *H. glycines* cysts placed on non-aerated and artificially aerated Baermann funnels.

Rather than restricted aeration, Wallace (27) suggested that products from cysts that inhibit larval emergence might accumulate in a hatching medium of greater depth.

Desiccation.—The effect of air drying of cysts on larval emergence was studied. In two tests, involving replicate samples of 500 cysts, and a third test of 12 replications of 100 cysts, cysts were placed in open Petri dishes. Excess water was carefully removed from the Petri dishes and they were then placed in a current of forced air to hasten the elimination of free moisture. Subsequently, the samples were stored at random in an incubator maintained between 25° and 30°C and a relative humidity between 40 and 50%. The Petri dishes were removed periodically and the cysts suspended in distilled water. The cysts were collected on an 80-mesh sieve and carefully washed onto Scotties tissue on a Baermann funnel. Ten and 20 days after placement of the cysts on the Baermann funnel, larvae were collected and counted.

Desiccation of cysts in open Petri dishes greatly reduced the emergence of larvae. As the period of desiccation increased, the number of larvae collected decreased. Larval emergence, as represented by larval collections, was reduced 97% after desiccation of cysts for one week. Few larvae emerged after desiccation of cysts for 2 months and larvae were not collected after desiccation of cysts for 77 days. Results are summarized in Fig. 2-B. Microscopic observations on the contents of the desiccated cysts indicated that many of the eggs contained dead larvae. Attempts failed to stimulate larval emergence by exposing cysts to various temperatures and soybean-root diffusate.

In similar tests at room temperature, Skotland (22) reported that the emergence of larvae from cysts of *H. glycines* was greatly reduced by exposure to air-drying for 30–71 days. The relative percentage of larvae that emerged from cysts exposed to air drying for 365 days, however, was similar to that reported for the shorter period of desiccation. Epps (8) reported that *H. glycines* cysts and cysts mixed with soil and stored in bags of soybean seed lacked viable contents after one month. Cysts mixed with soil and debris and exposed to air-drying in clay saucers lacked viable contents after 2 months. Drying of soil and cyst material in the collection, purification, and storage of *H. rostochiensis* cysts before hatching tests is an accepted practice (11). However, Lewis and Mai (18) reported considerable loss in viability of *H. rostochiensis* cyst contents exposed to the extreme relative humidities of 1.5 and 88%. Working with *H. schachtii* cysts, Wallace (25) reported that larval emergence was reduced by exposure of cysts to a high "saturation deficiency." Pretreatment of cysts by exposure to a "low saturation deficiency," however, increased larval emergence. Exposure of *H. major* cysts to slight desiccation greatly reduced larval emergence (14, 28). Franklin (12) reported that larvae failed to emerge from cysts of *H. punctata* Thorne that had been dried.

Discussion.—From the results, it is apparent that various environmental factors affect and regulate the emergence of larvae from cysts of *Heterodera glycines*. Undoubtedly these factors are also operative in nature; however, no attempt is made to relate the laboratory results directly to field conditions, where the biotic and abiotic environment is more complex. The results should serve as a basis for further work with

H. glycines as well as provide data on its behavior.

It is apparent that similarities and differences exist between *H. glycines* and other *Heterodera* species in the influence of environmental factors on larval emergence. As pointed out by Wallace (27), the tendency to apply general behavior patterns of one nematode to another, even within the genus *Heterodera*, is unwise.—University of Arkansas, Fayetteville.

LITERATURE CITED

1. Baunacke, W. 1922. Untersuchungen zur Biologie und Bekämpfung des Rübennematoden Heterodera schachtii Schmidt. Arb. biol. Reichsanstalt Land-u. Forstwirtsch. Berlin Dahlem 11:185-288.
2. Bishop, D. D. 1953. Hatching the contents of cysts of Heterodera rostochiensis with alternating temperature conditions. Nature, London. 172:1108.
3. Boyd, A. E. W. 1943. Observations on the biology of the potato-root eelworm, Heterodera schachtii Schmidt. Ann. Appl. Biol. 30:157-161.
4. Den Ouden, H. 1954. Het bietencystenaaltje en zijn bestrijding. I. Methoden te gebruiken bij het onderzoek naar kunstmatige en natuurlijke lokstoffen. Mededel. Inst. Rationele Suikerprod. 24:101-121.
5. Duggan, J. J. 1959. On the number of generations of beet eelworm, Heterodera schachtii Schmidt, produced in a year. Nematologica 4:241-244.
6. Ellenby, C. 1955. The seasonal response of the potato-root eelworm Heterodera rostochiensis Wollenweber: Emergence of larvae throughout the year from cysts exposed to different temperature cycles. Ann. Appl. Biol. 43:1-11.
7. Ellenby, C. 1956. The cyst of the potato-root eelworm (Heterodera rostochiensis Wollenweber) as a hatching unit. Ann. Appl. Biol. 44:1-15.
8. Epps, J. M. 1958. Viability of air-dried Heterodera glycines cysts. Plant Disease Reptr. 42:594-595.
9. Feldmesser, J., and G. Fassuliotis. 1950. Reactions of the golden nematode of potatoes, Heterodera rostochiensis Wollenweber, to controlled temperatures and to attempted control measures. J. Wash. Acad. Sci. 40:355-361.
10. Fenwick, D. W. 1951. Investigation on the emergence of larvae from the cysts of the potato-root eelworm, Heterodera rostochiensis. 4. Physical conditions and their influence on larval emergence in the laboratory. J. Helminthol. 25:37-48.
11. Fenwick, D. W., and E. Widdowson. 1958. The conduct of hatching tests on cysts of the potato-root eelworm Heterodera rostochiensis (Woll). J. Helminthol. 32:125-134.
12. Franklin, M. T. 1940. On the identification of strains of Heterodera schachtii. J. Helminthol. 18:63-84.
13. Hamblen, M. L., and D. A. Slack. 1959. Factors influencing the emergence of larvae from cysts of Heterodera glycines Ichinohe. Cyst development, condition, and variability. (Abstr.) Phytopathology 49:317.
14. Hesling, J. J. 1956. Some observations on Heterodera major. Nematologica 1:56-63.
15. Hesling, J. J. 1957. The hatching response of Heterodera major (O. Schmidt) to certain root diffusates. Nematologica 2:123-125.
16. Ichinohe, M. 1955. Studies on the morphology and ecology of the soybean cyst nematode, Heterodera glycines, in Japan (in Japanese, with English summary). Rept. Hokkaido Natl. Agr. Expt. Sta. 48:1-64.
17. Jones, F. G. W. 1950. Observations on the beet eelworm and other cyst-forming species of Heterodera. Ann. Appl. Biol. 37:407-440.
18. Lewis, Freda J. von Mechow, and W. F. Mai. 1960. Survival of encysted and free larvae of the golden nematode in relation to temperature and relative humidity. Proc. Helm. Soc. Wash. 27:80-85.
19. Lownsbery, B. F. 1950. Stimulation of golden nematode larvae by root leachings. (Abstr.) Phytopathology 40:18.
20. Lownsbery, B. F. 1951. Larval emigration from cysts of the golden nematode of potatoes, Heterodera rostochiensis Wollenweber. Phytopathology 41:889-896.
21. Shepherd, Audrey M. 1959. Increasing the rate of larval emergence from cysts in hatching tests with beet eelworm, Heterodera schachtii Schmidt. Nematologica 4: 161-164.
22. Skotland, C. B. 1957. Biological studies of the soybean cyst nematode. Phytopathology 47:623-625.
23. Slack, D. A., and M. L. Hamblen. 1959. Factors influencing the emergence of larvae from cysts of Heterodera glycines Ichinohe. Influence of constant temperature. (Abstr.) Phytopathology 49:319-320.
24. Triffitt, M. J. 1930. On the bionomics of Heterodera schachtii on potatoes, with special reference to the influence of mustard on the escape of the larvae from the cysts. J. Helminthol. 8:19-48.
25. Wallace, H. R. 1955. Factors influencing the emergence of larvae from cysts of the beet eelworm, Heterodera schachtii Schmidt. J. Helminthol. 29:3-16.
26. Wallace, H. R. 1955. The influence of soil moisture on the emergence of larvae from cysts of the beet eelworm, Heterodera schachtii Schmidt. Ann. Appl. Biol. 43:477-484.
27. Wallace, H. R. 1959. Further observations on some factors influencing the emergence of larvae from cysts of the beet eelworm Heterodera schachtii Schmidt. Nematologica 4:245-252.
28. Winslow, R. D. 1955. The hatching responses of some root eelworms of the genus Heterodera. Ann. Appl. Biol. 43:19-36.
29. Winstead, N. N., and C. B. Skotland. 1957. Eradicant treatments for narcissus bulbs and gladiolus corms harboring soybean nematode cysts. Phytopathology 47: 67-69.

PLANT INJURY INDUCED BY OZONE

A. C. Hill, M. R. Pack, M. Treshow, R. J. Downs, and L. G. Transtrum

Respectively, Supervisor of Research and Technology, Plant Physiologist, Plant Pathologist, Agronomist, and General Supervisor, Agricultural Division, Geneva Works, Columbia-Geneva Steel Division, United States Steel Corporation, Provo, Utah.

Accepted for publication November 7, 1960.

SUMMARY

Phytotoxicity of ozone to 34 plant species was studied in controlled-atmosphere greenhouses. Plants were subjected at various stages of growth to 0.13–0.72 ppm ozone for 2-hour periods. Injury symptoms developed on 28 species. Some of the most sensitive species were small grains, alfalfa, spinach, and tobacco. There was a general tendency for sensitivity to increase with maturity of tissue. Palisade cells were most readily injured by ozone. On plants with adaxial palisade parenchyma, chlorotic spots and bleached necrotic areas developed on the upper leaf surface. Injury was equally apparent from either leaf surface of plants with undifferentiated mesophyll. Necrotic spots extending completely through the leaf developed on plants with either mesophyll structure when injury was severe. Ozone caused conspicuous tumors to develop on broccoli leaves. Symptoms similar to those produced by ozone fumigations have been observed on a wide range of plant species growing near several large metropolitan centers.

Introduction.—High concentrations of ozone are associated with the Los Angeles type of smog, a type of air pollution becoming a problem in many metropolitan areas (5). According to Scott et al (17), elevated atmospheric ozone concentrations arise from photolysis of nitrogen dioxide in the presence of certain free organic radicals originating from incomplete combustion of organic materials. The photolysis is reversible, so ozone concentrations do not become high unless the free radicals are present to react with the nitric oxide produced, thereby preventing the back reaction. Sources of oxides of nitrogen and partially oxidized organic materials, i.e., automobiles and domestic and industrial furnaces, will undoubtedly continue to multiply with increased urbanization. Resultant increases in atmospheric ozone may have considerable effect on agriculture near metropolitan centers.

Most plant injury from smog has been attributed to other components of the smog complex, including reaction products of ozone and organic compounds (4, 6, 12, 13, 14), but direct injury by ozone has recently been recognized as an important air pollution problem (3, 7, 15, 16). Progress on identifying and evaluating this problem has been hampered by lack of information on the symptomatology of ozone injury.

The phytotoxicity of ozone was recognized as early as 1914, when Knight and Priestly (10) reported injury produced in the laboratory on Brussels sprouts, pea, wheat, and rye. Homan (9) described necrotic speckling on leaves of bean fumigated with ozone. Haagen-Smit et al (6) produced injury on spinach, endive, and alfalfa by exposure for 5 hours to 0.20 ppm ozone. Recently, Ledbetter, et al (11) reported investigations of ozone effects on a variety of plants.

In 1954, the Stanford Research Institute (18) described upper-surface glazing, a symptom characteristic of ozone injury, on spinach and other crops in the Los Angeles area. In 1956, Middleton (12) reported occasional field occurrence of ozone injury on bean, but did not consider it of economic importance. Ozone was first recognized as a phytoxicant of economic importance when Richards, et al (15, 16) showed that it caused grape stipple in the San Bernardino Valley. In 1959, Heggestad and Middleton (7) reported investigations indicating that ozone was the cause of "weather fleck" of tobacco, a serious problem in the production of cigar-wrapper tobacco in the Connecticut Valley. In 1959, Daines et al (3) observed ozone-type injury on 12 different crops at various locations in New Jersey.

The investigation reported here was conducted primarily to determine the response to ozone of 34 plant species. Macroscopic symptoms and pathological histology of ozone injury were studied to facilitate accurate diagnosis in the field.

Materials and methods.—Ozone fumigation studies were conducted in 3 controlled-atmosphere greenhouses and in portable polyvinyl chambers over plants in field plantings. The facilities have been described in detail by Hill et al (8). One greenhouse was maintained as a control, and ozone treatments were applied in the other greenhouses at intervals of 1–2 weeks. Ozone was generated electrostatically from tank oxygen. The output of the generator was injected into the inlet duct of the greenhouse or chamber air-circulation system. Each fumigation was for 2 hours at a constant setting of the ozone generator.

Ozone concentrations were determined throughout each fumigation by drawing metered air samples through 2% unbuffered potassium iodide solution and measuring the liberated iodine titrimetrically with standard sodium thiosulfate as proposed by Birdsall et al (1). Each sample was collected over a 30-minute interval at 6 cu ft/hr using 2 all-glass midget impingers connected in series, each containing 20 ml of potassium iodide solution.

Most of the fumigations were conducted in the greenhouses. Identical plantings were made in each greenhouse in plots of a uniform soil mixture. Other plants, grown in pots, were placed in the greenhouses during certain fumigations. The portable chambers were used in established field plantings for all fumigations of grape, 1 of alfalfa, and 6 of spinach. Temperatures ranged from 66 to 91°F, and relative humidity from 32 to 59%, in the different fumigations.

Plants of 34 species representing 17 families were subjected to ozone (Table 1). Spinach was represented by 4 varieties and corn and bean by 2 each. Some species were studied extensively, e.g., spinach was fumigated 32 times at all stages of growth up to the harvest stage, in greenhouse and field plantings. Plants of several species were fumigated only 1–3 times, to determine if the injury followed the general pattern seen on other species.

Symptom development was observed closely, and photographs and detailed descriptions were made of the macroscopic evidence of injury. Histological effects of the ozone treatments were studied, using freehand, unstained sections of fresh material.

Results.—*Sensitivity to ozone.*—Table 1 shows the range of ozone concentrations used on plants of each species and the lowest concentration that caused injury. Injury symptoms were produced on 28 of the 34 species fumigated. The results did not provide critical interspecific comparisons of ozone sensitivity, because different ozone concentration ranges were used, the plants were treated at different stages of growth, and most species were represented by a single variety. Some broad general differences in sensitivity were obvious, however.

Table 1.—List of plants studied, showing concentration range of ozone treatments and lowest concentration that produced injury with 2-hour exposure.

Family, species, and variety	Ozone concentration[a] (ppm)
Gramineae	
Annual bluegrass (*Poa annua* L.)	0.13–0.64 (0.64)
Orchard grass (*Dactylis glomerata* L.)	0.35–0.41 (0.35)
Wheat (*Triticum aestivum* L.), Lemhi	0.13–0.41 (0.23)
Oat (*Avena sativa* L.), Overland	0.13–0.41 (0.23)
Barley *Hordeum vulgare* L.), Bonneville	0.13–0.41 (0.23)
Rye (*Secale cereale* L.)	0.13–0.41 (0.23)
Field corn (*Zea mays* L.), Portwalco 100	0.13–0.41 (0.25)
Sweet corn (*Zea mays* L.), Iochief	0.13–0.41 (0.25)
Amaryllidaceae	
Onion (*Allium cepa* L.), Yellow Sweet Spanish	0.34–0.48 (NI)[b]
Leguminosae	
Alfalfa (*Medicago sativa* L.), Ranger	0.13–0.48 (0.20)
Red clover (*Trifolium pratense* L.)	0.35–0.41 (0.35)
Bean (*Phaseolus vulgaris* L.), Black Valentine	0.13–0.64 (0.25)
Mexican Pinto	0.13–0.64 (0.25)
Chenopodiaceae	
Beet (*Beta vulgaris* L.), Detroit Dark Red	0.13–0.41 (0.41)
Spinach (*Spinacia oleracea* L.), Old Dominion	0.13–0.64 (0.23)
Virginia Savoy	0.13–0.64 (0.23)
Bloomsdale Long Standing	0.13–0.64 (0.23)
Hybrid 7	0.13–0.64 (0.23)
Cruciferae	
Broccoli (*Brassica oleracea botrytis* L.), Italian Green Sprouting	0.25–0.41 (0.25)
Turnip (*Brassica rapa* L.), Purple-Top White Globe	0.35–0.41 (0.35)
Radish (*Raphanus sativus* L.), Sparkler, White Tip	0.35–0.41 (0.35)
Umbelliferae	
Parsnip (*Pastinaca sativa* L.), White Model	0.35–0.41 (0.35)
Carrot (*Daucus carota* L.), Imperator	0.35–0.41 (0.35)
Parsley (*Petroselinum hortense* Hoffm.), Moss Curled	0.13–0.41 (0.28)
Solanaceae	
Tomato (*Lycopersicon esculentum* Mill.), V. R. Moscow	0.13–0.41 (0.25)
Tobacco (*Nicotiana tabacum* L.), Variety "C"[c]	0.16–0.48 (0.24)
Petunia (*Petunia hybrida* Vilm.), Rose Charm	0.34–0.48 (0.34)
Cucurbitaceae	
Cucumber (*Cucumis sativus* L.), Marketer	0.13–0.41 (NI)[b]
Malvaceae	
Cotton (*Gossypium hirsutum* L.), Upland 1517	0.35–0.41 (NI)[b]
Compositae	
Lettuce (*Lactuca sativa* L.), Romaine	0.35–0.41 (NI)[b]
Endive (*Cichorium endivia* L.), Green Curled	0.35–0.41 (0.35)
Chrysanthemum (*Chrysanthemum* sp.)	0.41 (0.41)
Rosaceae	
Peach (*Prunus persica* L.), Elberta	0.13–0.41 (0.28)
Vitaceae	
Grape (*Vitis vinifera* L.), Mission	0.34–0.72 (0.34)
Begoniaceae	
Begonia (*Begonia* sp.)	0.41 (0.41)
Geraniaceae	
Geranium (*Geranium* sp.)	0.34–0.48 (0.41)
Labiatae	
Coleus (*Coleus blumei* L.)	0.41 (NI)[b]
Onagraceae	
Fuchsia (*Fuchsia* sp.)	0.41 (0.41)
Balsaminaceae	
Impatiens (*Impatiens* sp.)	0.41 (NI)[b]

[a] Range of fumigations and (in parentheses) lowest concentration that produced injury.
[b] NI means "no injury."
[c] Fleck susceptible variety from the Connecticut Valley, obtained from H. E. Heggestad, Beltsville, Maryland.

In Table 2, the plants are divided into 3 ozone sensitivity classes. The plants in the sensitive class include those injured at ozone concentrations of less than 0.30 ppm and some not fumigated at less than 0.30 ppm that developed such severe symptoms at slightly higher concentrations their sensitivity was evident. Plants in the intermediate class were not appreciably injured unless the concentration exceeded 0.30 ppm. Those in the resistant class were not injured by less than 0.40 ppm ozone, and some were not injured at all. Five ornamental species were not classified. The single fumigation given them was inadequate for evaluation of their sensitivity.

Wide differences in sensitivity to ozone were found between different species within the same family, e.g., spinach and beet, but no intervarietal differences were noted with spinach, corn, or bean.

There was a definite relationship between maturity

Table 2.—Sensitivity of plants to injury by ozone.

SENSITIVE
Spinach (*Spinacea oleracea* L.)
Tobacco (*Nicotiana tabacum* L.)
Alfalfa (*Medicago sativa* L.)
Wheat (*Triticum aestivum* L.)
Oat (*Avena sativa* L.)
Barley (*Hordeum vulgare* L.)
Rye (*Secale cereale* L.)
Orchard grass (*Dactylis glomerata* L.)
Red clover (*Trifolium pratense* L.)
Radish (*Raphanus sativus* L.)
Bean (*Phaseolus vulgaris* L.)
Corn (*Zea mays* L.)
Tomato (*Lycopersicon esculentum* Mill.)
Broccoli (*Brassica oleracea botrytis* L.)
Parsley (*Petroselinum hortense* Hoffm.)
INTERMEDIATE
Grape (*Vitis vinifera* L.)
Turnip (*Brassica rapa* L.)
Parsnip (*Pastinaca sativa* L.)
Carrot (*Daucus carota* L.)
Petunia (*Petunia hybrida* Vilm.)
Endive (*Cichorium endiva* L.)
Peach (*Prunus persica* L.)
RESISTANT
Beet (*Beta vulgaris* L.)
Geranium (*Geranium* sp.)
Annual bluegrass (*Poa annua* L.)
Onion (*Allium cepa* L.)
Cucumber (*Cucumis sativus* L.)
Cotton (*Gossypium hirsutum* L.)
Lettuce (*Lactuca sativa* L.)

of tissue and its sensitivity to ozone. The mature leaves were generally most severely injured, and the youngest leaves showing injury were injured at the tip end. Injury of pinnately compound leaves was most severe on the tip leaflet.

There were indications that environmental factors and the succulence of plants affect response to ozone, but these factors were not evaluated.

Symptomatology.—1) *General.*—Symptoms of ozone injury varied among different plant species, but certain general characteristics applied to most plants studied. The injury was generally characterized by numerous discrete lesions scattered over all or a large portion of the leaf. Depending on plant species and injury severity, the lesions ranged from small superficial spots to large necrotic areas.

For convenience of discussion, ozone-injury lesions may be classified into 3 general types: chlorotic mottling, necrotic bleaching of the upper leaf surface, and bifacial necrotic spots that extend completely through the leaf. The primary chlorotic lesions were usually less than 1 mm in diameter, but frequently developed in dense concentrations and merged into large, mottled, chlorotic spots. On leaves with adaxial palisade parenchyma, chlorotic lesions were largely confined to the upper surface, but were usually visible from the undersurface by transmitted light. Considerable chlorosis extended through to the lower surface on thin leaves. Superficial chlorotic lesions developed on either surface of leaves with undifferentiated mesophyll, but usually extended completely through the leaf.

Necrotic bleaching of the upper surface was a common symptom on leaves of plants with well-developed adaxial palisade parenchyma. The lesions ranged from a few cells in the center of a chlorotic spot to general bleaching of a large area of the leaf surface. Stippling or flecking of the upper surface with numerous small spots of this type was common on several plant species. Upper-surface necrosis was usually white or light tan, but dark pigments accumulated in the stipples on some species. On plants with undifferentiated mesophyll, injury that was severe enough to cause obvious cell necrosis usually produced lesions that extended completely through the leaf.

The bifacial necrotic spots were generally more than 1 mm in diameter, and sometimes the entire leaf was killed. Necrosis frequently extended through the leaf in a small area of a larger spot of upper-surface necrosis. Color of the necrotic spots ranged from ivory to light brown, depending on plant species.

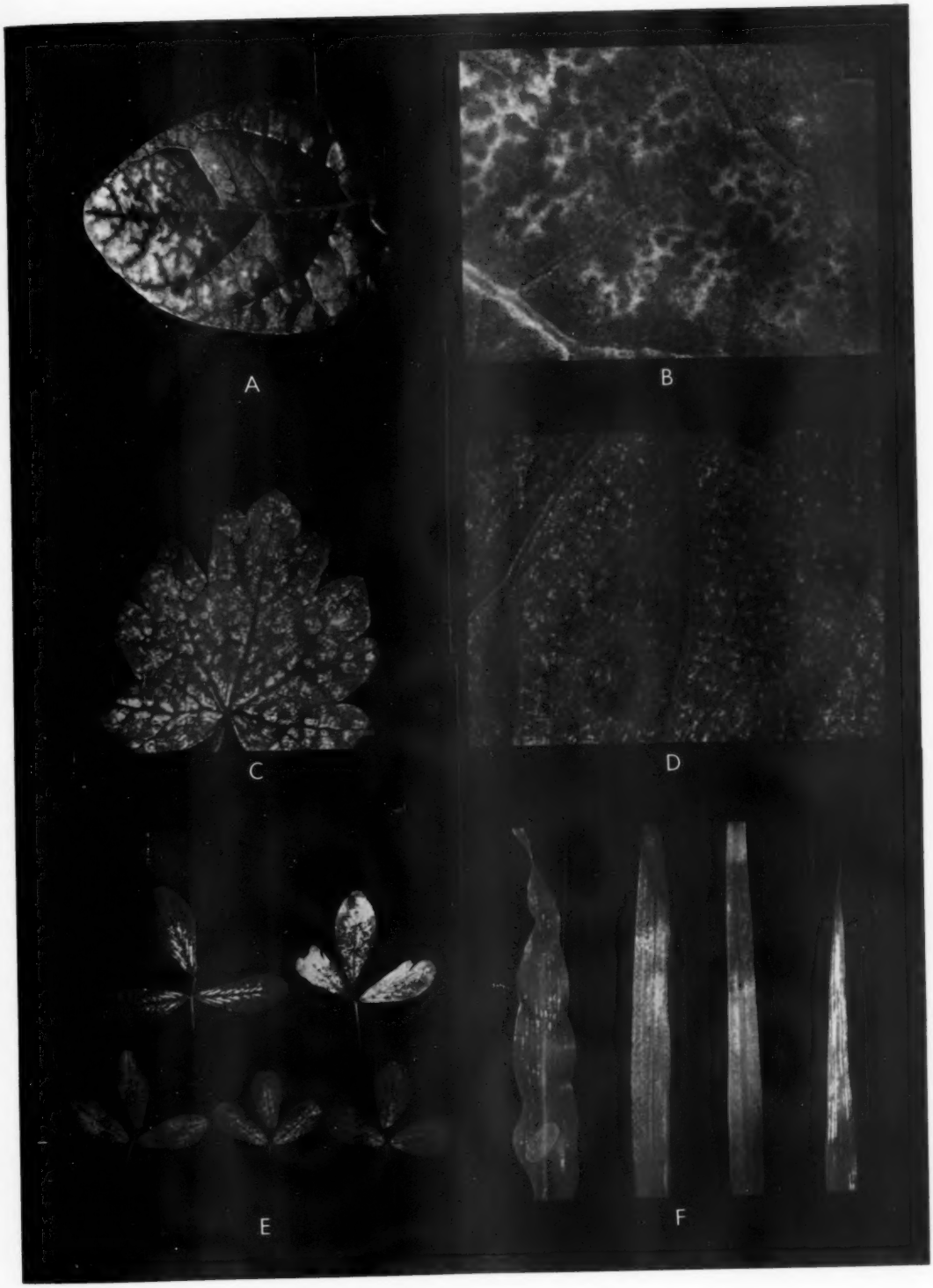
Large veins were unaffected by ozone unless injury was very severe, and the largest veins usually remained green even after most of the leaf tissue had collapsed. Lesions frequently concentrated along the sides of veins, however. Chlorosis and mild necrotic bleaching of the upper leaf surface often developed directly over small veinlets, forming a reticulate pattern.

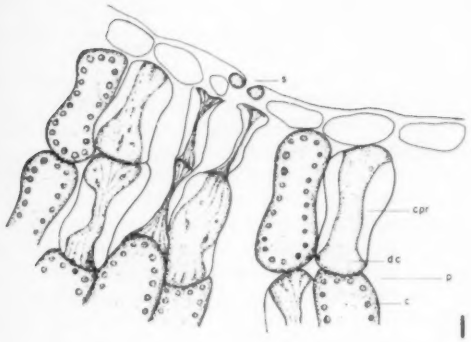
2) *Pathological histology.*—Palisade cells were much more prone to ozone injury than other tissues. Visible cellular changes accompanying chlorotic mottling and necrotic bleaching of the upper leaf surface occurred mainly in palisade cells. When 2–3 palisade layers were present, the outermost was usually, but not invariably, the first to show injury. On leaves without palisade cells, the outer spongy mesophyll cells were most readily affected.

The only apparent changes with chlorotic symptoms were disrupted chloroplasts and reduced chlorophyll content of affected cells. With necrotic bleaching, breakdown of the chloroplasts was accompanied by plasmolysis of the protoplast, but plasmodesmatal connections were apparently retained (Fig. 1). Brown pigments sometimes accumulated in affected cells of bean prior to collapse, forming dark stipples (Fig. 2). Cell wall collapse followed breakdown of the protoplast, but connection was retained to the cell immediately above or below (Fig. 3). Collapse of the cells left large intercellular spaces, which probably gave

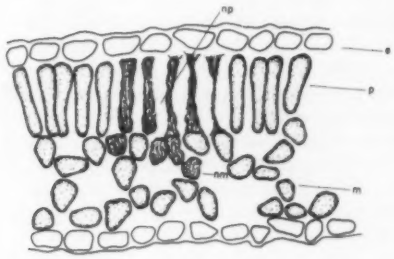
COLOR PLATE →

Fig. 7.—**A)** Typical ozone injury symptoms on spinach. **B)** Chlorosis and necrotic bleaching of upper surface of broccoli leaf. **C)** Chlorosis and necrotic bleaching of upper surface of parsnip leaf. **D)** Close-up of lesions on bean leaf. **E)** A range of symptoms on alfalfa. **F)** Ozone injury symptoms on, left to right, corn, wheat, barley, and oat.

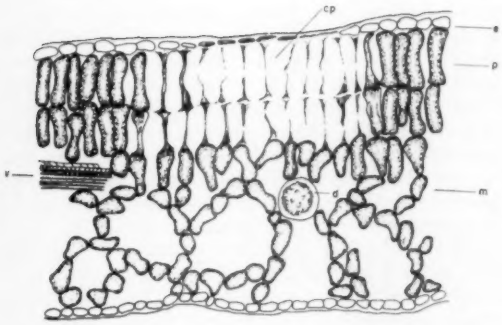

A
B
C
D
E
F



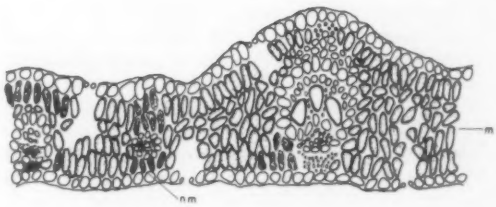
1



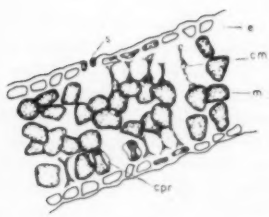
2



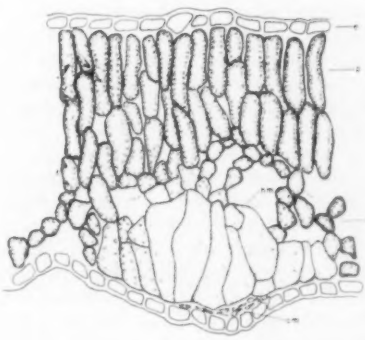
3



4



5



6

Fig. 1–6.—Camera lucida drawings of sections of leaves injured by fumigation with 0.28–0.41 ppm ozone. **1)** Injured spinach palisade tissue (p) with collapsing protoplast (cpr) and disintegrating chloroplasts (dc) in injured cells. More severe injury is shown in cells below stoma (s). Normal chloroplasts (c) are shown in uninjured cells. **2)** Bean leaf with necrotic palisade tissue (np) containing pigments. In the underlying mesophyll (m) a few cells are necrotic (nm). Epidermis (e) has remained normal over affected area. **3)** Cross section of spinach leaf showing collapsed palisade tissue (cp) overlain by collapsed epidermal tissue. Normal epidermis (e), palisade (p), and spongy mesophyll (m) cells, druse (d), and a section of vascular strand (v) are also shown. **4)** Wheat leaf section showing necrotic mesophyll (nm) primarily near small vascular bundles. **5)** Endive leaf section showing collapse of outer layers of mesophyll (cm) with central mesophyll (m) remaining normal. Early collapse of protoplast (cpr) precedes collapse of cell walls. **6)** Broccoli leaf section showing small tumor with hypertrophied spongy mesophyll cells (hm) which have crushed some of lower mesophyll cells (cm) against the still normal epidermis. Surrounding mesophyll cells (m) appear normal.

the affected tissue its white appearance. Small upper-surface flecks or stipples were produced by collapse of islands of 1 to several cells (Fig. 1, 2). Epidermal tissue, including the stomatal guard cells, overlying injured palisade cells generally remained normal unless a large area of palisade tissue was killed. The underlying spongy mesophyll also remained normal in most species when ozone injury was mild. When large areas were bleached, injury extended progressively into the spongy mesophyll.

Cells throughout the affected area appeared to collapse simultaneously to form the bifacial necrotic lesions. The adaxial and abaxial surfaces were drawn close together by the desiccating mesophyll, forming a thin, papery lesion. Vascular tissues were rather resistant to ozone injury, but when injury was severe, vascular tissue was killed and the necrotic areas included many small veins.

On plants of the *Gramineae*, which lack well-defined palisade tissue, the outer mesophyll adjacent to the vascular bundle sheath was most readily injured (Fig. 4). Cells overlying small vascular strands without bundle sheath extensions appeared to be especially sensitive to ozone. Superficial lesions on endive, which also lacks palisade cells, affected the outer mesophyll cells at either leaf surface (Fig. 5).

Microscopic examination of tumefaction that developed on broccoli leaves showed that swelling began in spongy mesophyll cells immediately below the palisade layer (Fig. 6). Subsequently, lower spongy mesophyll cells enlarged, and palisade cells were occasionally involved. No evidence of hyperplasia was seen. Hypertrophy of the central mesophyll cells adjacent to necrotic tissue was observed microscopically on tomato, but was not visible macroscopically. This type of response was observed only on broccoli and tomato.

3) *Vegetable crops.*—a) *Spinach.*—The complete range of symptoms, from mild chlorotic mottling of the upper surface to necrotic collapse of most of the leaf, was produced on spinach (Fig. 7-A). Frequently, all of the different symptoms developed on the same leaf. The first evidence of ozone injury, a shiny, oily appearance of the upper leaf surface, developed within 1–2 hours of beginning of exposure to injurious concentrations of ozone. After the ozone treatment was discontinued, many areas lost their oily appearance and returned to normal. Where permanent injury occurred, the leaf assumed a dull gray-green appearance. The tissue then appeared water-soaked in areas where the injury extended completely through the leaf, or bleached, giving chlorotic or necrotic lesions on the upper surface. Chlorosis generally formed a punctate pattern on the upper surface of the leaf. Two variations of the necrotic bleaching of the upper surface were observed. The more common was dull white, with affected areas collapsing to form slight depressions. Less frequently, the surface had a glazed, pearly appearance and the injury was very shallow with little or no apparent collapse of the affected area. After weathering a few days, the lesions frequently turned yellow or light tan. Unless the injury was severe enough to kill a large area, any bifacial necrotic spots that developed were usually scattered randomly over much of the leaf. Lesions usually were formed near the center of an interveinal area, and a band of tissue bordering the large veins often remained unaffected.

b) *Beet.*—Ozone injury was produced on beet only once, at the highest concentration used on this species (0.41 ppm). The symptoms were very similar to those of mild injury on spinach: chlorotic stippling and mild necrotic flecking of the upper surface of the leaf. There was a tendency for the lesions to form a reticulate pattern by following small veins. Accumulation of anthocyanins in some parts of the affected tissue gave the lesions a mottled red and tan color.

c) *Radish and turnip.*—Symptoms on radish and turnip (Fig. 8-C) resembled those on spinach. Chlorosis and necrotic bleaching were uniform over large areas of the upper surface of the leaf, extending close to the veins, most of which remained green. Necrosis extending through the leaf was more localized than on spinach, frequently concentrating in a particular area. Injury was generally most severe toward the leaf tip, but was sometimes concentrated along the midrib. Occasionally, a localized area of injury occurred at the apex of a bend in the leaf.

d) *Broccoli.*—Mild ozone injury to broccoli first appeared as light green chlorosis of the upper leaf surface along the small veins. The chlorosis gradually extended over the interveinal areas and was interspersed with necrotic bleaching, giving the upper surface of the leaf a mottled, yellow-green to white cast (Fig. 7-B). Bifacial necrotic spots (Fig. 8-A) occurred in localized areas as on radish and turnip, often concentrating along the midrib. In early stages of this type of injury, the upper surface of affected areas became lighter green and slightly depressed, but collapse was most pronounced on the underside of the leaf. The spots bleached to a light tan to buff.

Hypertrophy of tissues on the underside of the leaf beneath upper-surface chlorotic or necrotic areas appeared to be a specific symptom on broccoli. Initially, small irregular depressions appeared on the lower surface. Four to five days later, tumefaction became apparent in these areas, and swelling continued for several days, forming glandular protuberances up to about 1 mm in height. Individual protuberances were somewhat hemispherical, but frequently merged to form irregular shapes (Fig. 8-B). A similar but much milder condition sometimes developed on the upper surface at the margins of chlorotic and bleached areas. Hypertrophy sometimes developed on the underside of the leaf around the edges of bifacial necrotic spots, but this rarely occurred when the necrosis extended close to large veins.

e) *Parsnip.*—The most characteristic feature of ozone injury to parsnip was a rather uniform chlorotic to necrotic speckling of the upper leaf surface (Fig. 7-C). Some necrotic spots penetrated through the leaf. Upper-surface spots were sometimes centered

on small veinlets that remained green. The necrotic tissue was usually white to buff, but scattered dark brown spots were frequent.

f) *Parsley.*—Symptoms on parsley differed from those on most other plants in that there was greater tendency for development of chlorotic interveinal spots visible from the underside of the leaf. The light yellow-green chlorosis contrasted sharply with adjoining normal green areas, giving the leaf a variegated appearance. Injury was most severe near the tips of leaf lobes where white to buff necrotic spots 2–3 mm in diameter were common.

g) *Carrot.*—Symptoms on carrot were very similar to those on parsley except the lesions often developed at the leaf margins (Fig. 8-D). Upper-surface lesions were interveinal and were usually apparent from the undersurface. Bifacial necrotic spots were tan and membranous.

h) *Tomato.*—Fine, chlorotic stippling developed on the upper surface of tomato leaves with mild ozone injury. More severe injury resulted in general chlorosis of much of the leaf and areas of necrotic bleaching on the upper surface. Bifacial necrotic spots also developed, and sometimes a few scattered buff to brown spots were the only evidence of injury. When necrotic spots were numerous, they frequently concentrated along the leaflet midrib.

i) *Bean.*—In general, the expression of ozone injury was the same on both varieties of bean tested, but they sometimes responded differently to a particular fumigation. The typical symptom, which developed 1–2 days after fumigation, was necrotic flecking of the upper leaf surface (Fig. 7-D, 8-E). Usually, the flecks were light yellow-green to white, but affected cells sometimes filled with red-brown pigments. Along large veins there was usually a narrow band without markings. No lesions were produced that extended through to the undersurface of the leaf.

j) *Endive.*—Since endive has no palisade cells, ozone did not affect the upper surface preferentially. Symptoms first appeared as small, water-soaked spots, on either side of the leaf or extending through it. Within 2–3 days, the spots became light tan. Necrosis was usually most prevalent along the sides of major veins, especially the midrib.

4) *Field crops.*—a) *Alfalfa and red clover.*—Within a few hours of fumigation, injured areas of alfalfa leaves became dull, gray-green. Symptoms were of all 3 types (Fig. 7-E). The chlorosis was light yellow-green and, in mild cases, tended to concentrate along veins. When severe, the chlorosis extended over most of the interveinal tissue and was very apparent from the undersurface of the leaf. Frequently, numerous small islands of normal green tissue were scattered throughout the chlorotic areas. These green spots were up to 1 mm in diameter, fairly circular, and sharply defined. Necrotic bleaching of the upper leaf surface was silver-gray to tan. It occasionally occurred in small spots but usually spread over a fairly large area, affecting several adjacent interveinal areas. Bifacial necrotic spots usually concentrated along the midrib or secondary veins. Large necrotic areas formed at various locations, including the tip, margins, and base of the leaf. Occasionally an entire leaf was killed.

Symptoms on red clover were similar to those on alfalfa, but leaves had greater tendency to collapse completely. Necrotic tissue on red clover was light red-brown, compared to light tan on alfalfa.

b) *Small grains and grasses.*—Symptoms were similar on barley, oat, rye, wheat, orchard grass, and annual bluegrass (Fig. 7-F). The mildest symptom was fine chlorotic stippling between the large veins. When injury was more severe, chlorotic interveinal streaks developed, apparently from merging of numerous smaller lesions. Since these species lack clearly-defined palisade cells, mild superficial lesions developed on either leaf surface, but moderate to severe lesions extended completely through the leaf.

Necrotic lesions appeared as light tan to ivory streaks or oblong spots, extending lengthwise on the leaf and often affecting the smallest veins. Necrosis was frequently concentrated in a particular area, such as at the apex of a bend in a leaf, where it sometimes extended almost across the leaf except for the largest veins. On seedlings, necrotic areas were frequently most prevalent at the margins and tip of the leaf. Oval spots or broad streaks of necrosis, extending across the secondary veins, formed at the leaf margins and tips of annual bluegrass.

c) *Corn.*—Symptoms of ozone injury on corn were mostly necrotic streaks (Fig. 7-F). Chlorosis was evident only along the margins and at the ends of the streaks. Injury first appeared a few hours after fumigation as dull gray-green water-soaked areas.

d) *Tobacco.*—Symptoms of mild ozone injury on tobacco were light gray to tan flecks on the upper surface of the leaf (Fig. 8-F). These irregularly shaped flecks frequently formed a reticulate pattern by following the small veinlets. On close examination, the flecks appeared as small, sunken necrotic areas bordered by chlorotic tissue. The most inconspicuous flecks appeared to be only chlorotic. With severe injury, bifacial necrotic spots formed. These spots were generally concentrated along the sides of the midrib and large secondary veins.

5) *Fruit crops.*—a) *Grape.*—Symptoms on grape were similar to those on bean in which dark pigment accumulated in the affected palisade cells. Brown to black lesions about 1 mm in diameter were randomly scattered over the leaf but rarely transected any veins. With exposure to severe ozone treatment, large areas of the leaf collapsed and became necrotic.

b) *Peach.*—A mild chlorotic upper-surface mottling along veins was the only symptom produced on peach.

6) *Ornamental plants.*—Ozone injury symptoms were produced on petunia, chrysanthemum, fuchsia, begonia, and geranium. Symptoms on petunia were very mild, consisting of dull white upper-surface bleaching along leaf veins. Chrysanthemum developed light tan, necrotic speckling of the upper leaf surface similar to that on parsnip. Symptoms on fuchsia

and begonia were much alike, consisting of sunken upper-surface necrotic spots, some of which extended through the leaf. On geranium, ozone produced scattered intercostal necrotic spots that extended through the leaf (Fig. 8-C).

Discussion.—The rather distinctive symptoms produced by ozone, particularly on plants with well-developed palisade parenchyma, should facilitate identification of any significant occurrence of injury in the field. The most distinctive characteristics were the preponderance of upper-surface injury, the scattered distribution of fairly symmetrical, discrete lesions, and the tendency for lesions to develop over veinlets and along the sides of larger veins. Some symptoms

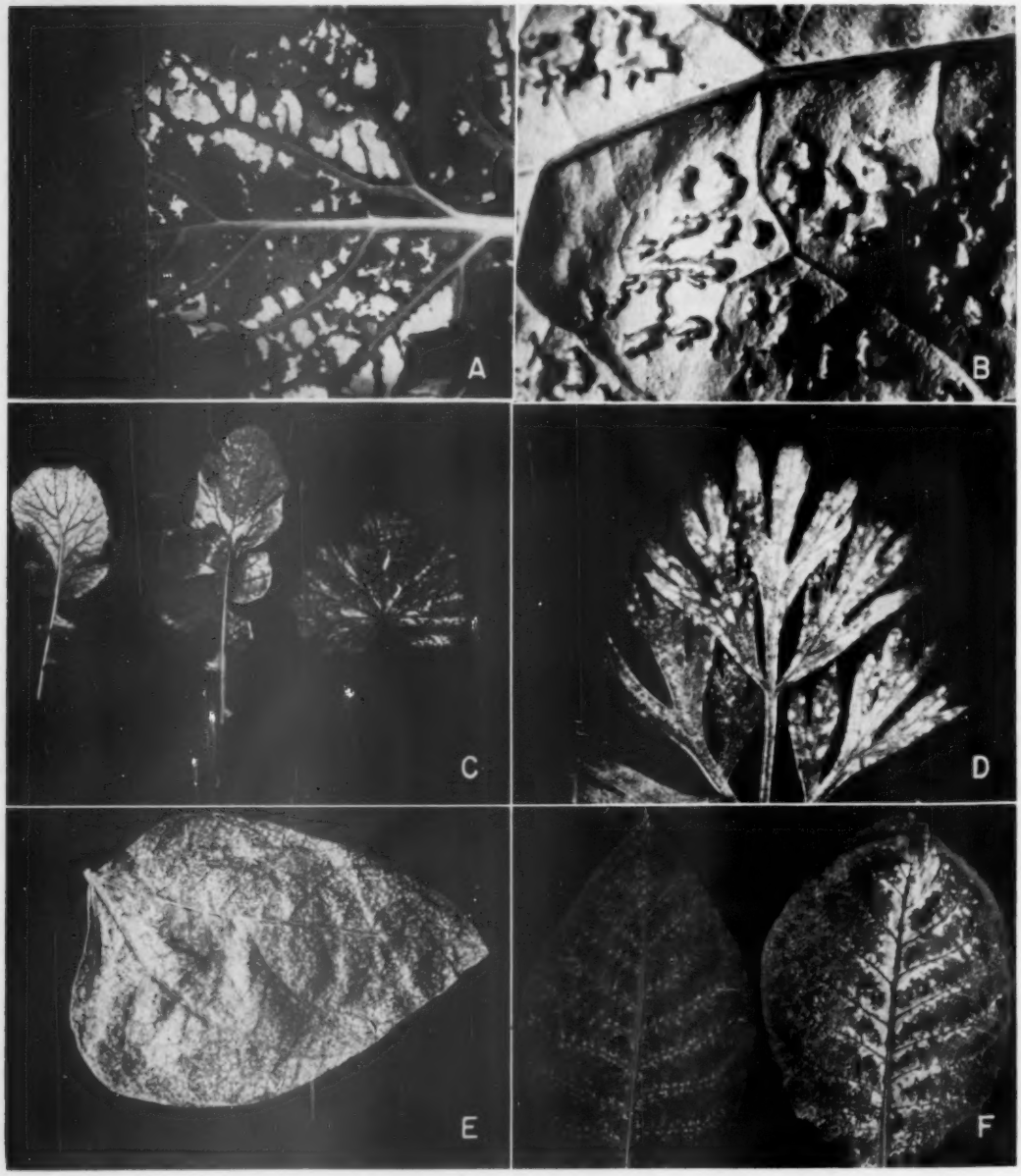


Fig. 8.—Ozone injury symptoms. **A)** Necrosis extending through broccoli leaf. **B)** Tumors on undersurface of broccoli leaf. **C)** Left to right, radish, turnip, and geranium. **D)** Bleached lesions on carrot. **E)** Stippling on upper surface of bean leaf. **F)** Flecking (left) and necrotic lesions (right) on tobacco.

were similar to those that may be produced by other causes, so difficulty might be encountered in diagnosing limited occurrences of ozone injury.

Histological effects of ozone contrasted sharply to those reported for ozone-hydrocarbon products. Palisade cells were most readily injured by ozone, whereas Bobrov (2) showed that ozonated hydrocarbons primarily affected the spongy mesophyll cells lining substomatal chambers. Since greater injury of palisade cells cannot be attributed to accessibility to the atmosphere, cells apparently exhibit differential sensitivity to ozone.

A high sensitivity of many plants to ozone was apparent from classification of 15 of the 34 species studied as "sensitive." Atmospheric ozone concentrations of the magnitude that injured these plants are not uncommon near large urban centers (6, 7). Symptoms indistinguishable from those produced by ozone fumigation have been observed by the authors near several large United States cities on a number of plants, including spinach, radish, parsley, bean, endive, alfalfa, red clover, tobacco, small grains, orchard grass, grape, and several weeds and ornamental species. Injury was sometimes distributed over a large geographical area, and typical Los Angeles smog-type "oxidant" injury, glazing or silvering of the undersurface of leaves, was usually found on sensitive species throughout the same area.

Although no differences in sensitivity to ozone were apparent among the varieties of corn, bean, or spinach studied, Heggestad and Middleton (7) reported differences in sensitivity of tobacco varieties, and Ledbetter et al (11) found varietal differences in tobacco and bean. Richards et al (15, 16) observed varietal differences in ozone injury severity on grape. Sensitivity to ozone may eventually influence the selection of crops for growth in certain areas, especially if the crop value is appreciably affected by appearance of the leaves.—Agricultural Division, Geneva Works, Columbia-Geneva Steel Division, United States Steel Corporation, Provo, Utah.

LITERATURE CITED

1. Birdsall, C. M., A. M. Jenkins, and E. Spadinger. 1952. Iodometric determination of ozone. Anal. Chem. 24:662-664.

2. Bobrov, Ruth A. 1952. The anatomical effects of air pollution on plants. Proc. 2nd National Air Pollution Symposium. Stanford Research Institute, Pasadena, Calif. p. 129-134.

3. Daines, R. H., Ida A. Leone, and Eileen Brennan. 1960. Air pollution as it affects agriculture in New Jersey. New Jersey Agr. Expt. Sta. Bull. 794. 14 p.

4. Darley, E. F., E. R. Stephens, J. T. Middleton, and P. L. Hanst. 1959. Oxidant plant damage from ozone-olefin reactions. Intern. J. Air Pollution (London) 1: 155-162.

5. Haagen-Smit, A. J. 1958. Air conservation. Science 128:869-878.

6. Haagen-Smit, A. J., E. F. Darley, M. Zaitlin, H. Hull, and W. Noble. 1952. Investigation on injury to plants from air pollution in the Los Angeles area. Plant Physiol. 27:18-34.

7. Heggestad, H. E., and J. T. Middleton. 1959. Ozone in high concentrations as cause of tobacco leaf injury. Science 129:208-210.

8. Hill, A. C., L. G. Transtrum, M. R. Pack, and A. Holloman, Jr. 1959. Facilities and techniques for maintaining a controlled fluoride environment in vegetation studies. J. Air Pollution Control Assoc. 9:22-27.

9. Homan, C. 1937. Effects of ionized air and ozone on plants. Plant Physiol. 12:957-978.

10. Knight, R. C., and J. H. Priestly. 1914. The respiration of plants under various electrical conditions. Ann. Botany (London) 28:135-161.

11. Ledbetter, M. C., P. W. Zimmerman, and A. E. Hitchcock. 1959. The histopathological effects of ozone on plant foliage. Contribs. Boyce Thompson Inst. 20: 275-282.

12. Middleton, J. T. 1956. Response of plants to air pollution. J. Air Pollution Control Assoc. 6:7-9, 50.

13. Middleton, J. T., E. F. Darley, and R. F. Brewer. 1958. Damage to vegetation from polluted atmospheres. J. Air Pollution Control Assoc. 8:9-15.

14. Noble, W. M. 1955. Pattern of damage produced on vegetation by smog. J. Agr. Food Chem. 3:330-332.

15. Richards, B. L., J. T. Middleton, and W. B. Hewitt. 1958. Air pollution with relation to agronomic crops: V. Oxidant stipple of grape. Agron. J. 50:559-561.

16. Richards, B. L., J. T. Middleton, and W. B. Hewitt. 1959. Ozone stipple of grape leaf. Calif. Agr. 13(12):4, 11.

17. Scott, W. E., E. R. Stephens, P. L. Hanst, and R. C. Doerr. 1957. Further developments in the chemistry of the atmosphere. Proc. Am. Petrol. Inst. 37(Sect. III): 171-183.

18. Stanford Research Institute. 1954. The smog problem in Los Angeles County. Western Oil and Gas Assoc., Los Angeles, Calif. p. 70-71.

POPULATION LEVELS OF PRATYLENCHUS PENETRANS AS INFLUENCED BY TREATMENTS AFFECTING DRY WEIGHT OF WANDO PEA PLANTS

James S. Dolliver

Present address: Department of Plant Pathology, University of Wisconsin, Madison.

Accepted for publication November 7, 1960.

Adapted from part of a Ph.D. thesis, Cornell University. The author is indebted to Professors D. G. Clark and W. F. Mai for valuable help during the investigation.

SUMMARY

The numbers of *Pratylenchus penetrans* in the root systems of Wando peas grown in greenhouse and controlled-environment chambers were determined after treatments affecting the dry weight of the pea plant. The ability of this nematode to reproduce was related to the physiological status of the plant it attacks. Treatments that reduced plant dry weight moderately (less favorable light and temperature, excision of plant parts, and limitation of nutrient supply) also resulted in significantly greater *P. penetrans* numbers than on check plants. Treatments that reduced plant dry weight more severely reduced nematode numbers. Removal of pods increased root system size and reduced *P. penetrans* numbers per root system. The increased nematode numbers from the treatments indicated intimate interaction among these parasites, their hosts, and the environment.

Introduction.—The concept that the physiological condition of a host plant is able to affect the ability of its nematode parasites to reproduce has been appreciated by most people working with nematodes. Nevertheless, more evidence is needed to support and expand this concept. Unfortunately, effects of environmental changes on plant-parasitic nematode populations have often been published with little or no mention of how the host plant differed with changes in treatment.

This study was made to develop methods for quantitative study of *Pratylenchus* spp. under greenhouse conditions, and to determine how treatments affecting the physiological status (as indicated by dry weight) of a suitable host plant affect the nematode numbers within the root system.

Review of Literature.—Certain concepts of the relations between nematodes and the plants they attack have received much emphasis and experimental support in the literature. These include: 1) a distinction between a plant's suitability as a host for a given nematode parasite and its susceptibility to disease caused by the same nematode's activities as a pathogen, 2) a realization that the factors responsible for a plant's low host suitability and/or high disease resistance are not the same for every plant-nematode combination, and 3) recognition that the size and health of the root system of a suitable host could set an upper limit on the number of nematodes it could support.

Along with this there have been ample indications that the nematode-plant relationships are even more complex. Selected examples are mentioned below.

Irregular results with soybean varieties considered resistant to *Meloidogyne* spp. led Taylor (17) to suggest that root-knot resistance varies with ecological conditions. Reproduction (11) and development (12) of *M. incognita* attacking lima bean roots were accelerated by increasing the concentration of potassium supplied to the plants. In contrast, Bird (1) found *M. javanica* developed faster in roots of tomato plants deficient in nitrogen, magnesium, iron, or potassium, than on plants grown in full nutrient solution. Populations of *P. penetrans* on sour cherries were significantly lowered after high potassium application (10).

Spring-sown alfalfa that tended to fail as a crop and had poor roots had about 1000 times the number of *Pratylenchus* spp. per gram of root as a fall-planted crop that developed into excellent stands (2). *Pratylenchus* spp. had no marked effect on growth of alfalfa and red clover in the greenhouse until after the second cutting (to 2 in. shoot growth) if given high initial nematode populations, and after additional cuttings if given lower inoculum numbers (3).

M. incognita developed from eggs to adult females in 17 days on tomato plants inoculated at 65 and 75 days old, and in 40 days on plants inoculated at 35 days old (13). Collins and Hagan (5) noted that in pineapple plants attacked by *Meloidogyne* spp., short roots generally have heavier nematode infestation than long roots.

Studies with *Anguina agrostis* (6), *Aphelenchoides oryzae* (8), and *Ditylenchus* spp. (7) have also illustrated close associations of the life cycles of nematodes with the growth cycles of host plants.

Materials and Methods.—Wando variety garden peas (*Pisum sativum* L.) were planted in 3-in. pots of steamed soil (1:1 sand-loam, pH 6.5) in 8-in. pots of the same soil. This pea variety is advertised as having heat tolerance, growing well in greenhouse and growth-chamber environments. Roots of these plants remained permeate through the soil in the 8-in. pots until harvest, and were easy to separate from the soil ball. This was in contrast to tomato plants, which rapidly became "pot-bound" in 8-in. pots, and were poor greenhouse hosts for the nematodes studied.

The pea seeds were disinfested with calcium hypochlorite solution (10 g/140 cc water, filtered, and filtrate used immediately) for 40 minutes before planting. This tended to restrict root nodulation, which otherwise interfered with effective root incubation.

When the pea roots approached a pot-bound condition within the 3-in. pots (about 2 weeks after planting), inoculation procedures were started. Roots of orchard grass (*Dactylis glomerata* L.) obtained from an infested sour cherry orchard served as the inoculum source. After these roots were washed in cold tap water and picked free of debris they contained a population of *Pratylenchus* spp. essentially free of other

nematode genera. Nearly all the nematodes keyed to species (15) were characterized as *P. penetrans* (Cobb). Tail shape in this population was quite variable, however, and a few specimens had annulated tail tips.

The 3-in. pots with the pea plants were removed from the 8-in. pots, leaving a cup-like depression in the soil. This cavity was then lined with a sample of chopped orchard grass roots. The 3-in. pot was then removed from its soil ball and the soil with the pea plant placed into the soil cavity in the 8-in. pot. Representative samples of the inoculum source were taken and incubated for characterization of the inoculum numbers.

To allow time for the development of at least one new generation of nematodes, the experiments were not terminated until at least 5 weeks after inoculation. At harvest, the pea roots were removed from the soil, washed in cold tap water, and placed in 20 cc of tap water in Petri dishes for incubation at 24°C. The entire root system was incubated, as variation was high among unbiased samples from a given root system. In cases where the fresh weight of the roots exceeded 2 g, more than one incubation dish was used. Under these conditions, nearly all the emerged nematodes were highly active.

Counts were made until nematode emergence from the incubated roots approached a maximum value (usually 3 counts). After each count the roots were reincubated with fresh tap water. Examination of the roots after staining with acid fuchsin in lactophenol revealed that eggs and nematodes remaining in the roots numbered less than 1% of the value obtained for maximum emergence.

Soil samples (250 cc) were processed by modifications of the methods of Christie and Perry (4) or Seinhorst (14).

Shoots and roots after incubation were dried at 40°C to constant weight. Shoot dry weights include weight of any materials removed during a given experiment.

Defoliation (Fig. 1 and 6).—In the first defoliation experiment (Fig. 1) half of the pea plants were defoliated 3 days after receiving an inoculum averaging 580 nematodes per plant. Defoliation involved removal of leaves, but not the leafy stipules of the pea plants, and was continued until harvest, 8 weeks after inoculation. Each value in Fig. 1 represents the mean of 6 replications.

In the second defoliation experiment (Fig. 6), defoliation was started 2 and 21 days after inoculation with an inoculum averaging 90 nematodes per plant and was continued until harvest, 6 weeks after inoculation. The values for the plants defoliated from 2 days represent means for 7 plants; the other values represent 8 plants each.

Nutrition (Fig. 2).—Half of 24 pea plants growing in 1:1 fine-coarse sand and watered with tap water received Hoagland's full nutrient solution (9). Harvest was 5 weeks after inoculation with an average of 4600 nematodes per plant.

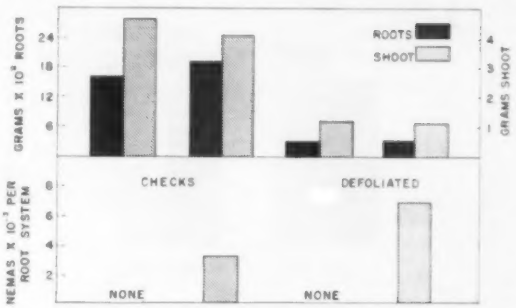


Fig. 1.—Effect of defoliation and *Pratylenchus penetrans* on the dry weight of Wando pea plants, and of defoliation on numbers of *P. penetrans* in pea root systems.

Controlled temperature and light intensity (Fig. 3).—After receiving inoculum averaging 120 nematodes per plant, 9 pea plants each were placed under continuous light at the intensities shown. Temperature was 77±4°F at the higher intensity, and 77±2°F at the lower intensity. Harvest was 5 weeks after inoculation.

Pod removal (Fig. 4).—Pods were removed from 24 of 48 pea plants 4 weeks after inoculation with an average of 130 nematodes per plant. Pod removal was continued until harvest, 8 weeks after inoculation.

Stem scalding (Fig. 5).—Peas were scalded at the bases of their stems 4 days after receiving an inoculum averaging 1100 nematodes per plant. The scalded stems and the stems of the checks were supported with aluminum-foil strips (½ in. wide) around the stem bases. In Fig. 5 the values for checks represent the means of 12 plants, those for scalded stems represent 9 plants. Harvest was 6 weeks after treatment.

Results.—Under the experimental conditions *P. penetrans* resulted in little or no change in the growth of Wando peas (Fig. 1), whether the plants were weakened or not.

Nematode numbers per root system were higher from plants weakened by defoliation (Fig. 1), from plants grown in quartz sand lacking supplemental nutrients (Fig. 2), from plants displaying reduced growth (as measured by dry weight) from being grown under light of lower intensity (Fig. 3). Nematode numbers per root system were lower from plants with root growth improved by removal of pods (Fig. 4).

In contrast, treatments severely restricting root growth reduced nematode numbers. The roots of plants with stems that had been scalded had less than half as many nematodes as check plants (Fig. 5). Nematode numbers declined as pea plants became senescent. Plants 12 weeks old and with 9/10 of their shoots necrotic had half as many *P. penetrans* per root system as plants 9 weeks old (3/10 necrotic).

The results of these experiments are best summarized by Fig. 6. When plant growth was severely restricted by early defoliation, nematode numbers were half those of the check plants. When restriction was only moderate because defoliation was later, twice as many nematodes were found per root system.

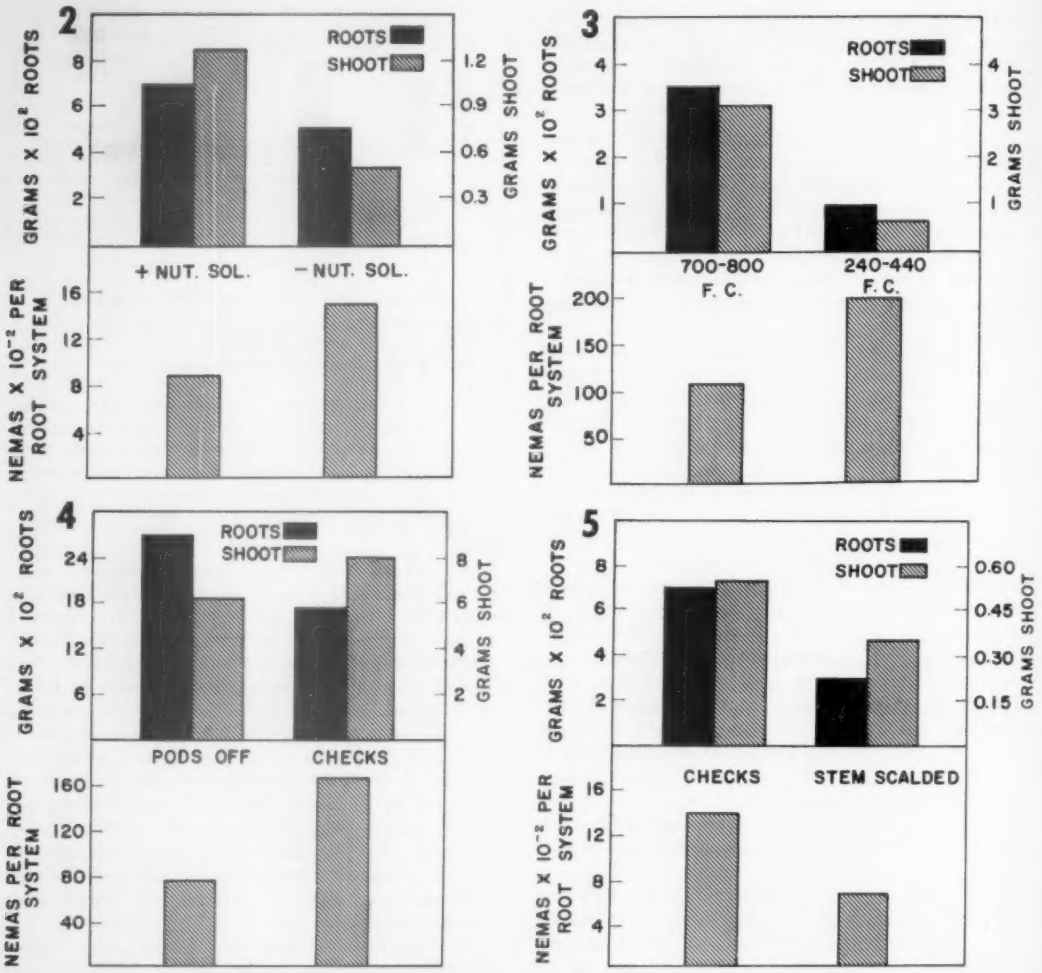


Fig. 2–5.—Effects of various treatments on numbers of *Pratylenchus penetrans* in root systems and dry weight of Wando peas. **2)** Addition of nutrient solution to peas grown in sand culture. **3)** Controlled temperature and light relationships. **4)** Pod removal. **5)** Stem scalding.

The results were analyzed with nonparametric methods as outlined by Snedecor (16). Statistical significance (at least 5% level) was obtained for differences in nematode numbers presented in Fig. 2, 3, 4, and 5.

No meaningful information was obtained from the soil samples processed, the nematode counts being very low (often only 1 or 2 nematodes) and highly variable.

The pea roots were able to grow away from the nematode inocula at the tops of the pots. In one experiment roots from the top halves of the pots had over 40 times as many nematodes as roots from the bottom halves.

Discussion.—Inadequate root growth can limit nematode populations within roots. Nematode populations were not maximum with best plant growth, however. Conditions that restricted plant growth moderately apparently caused "predisposition" of the pea plants for nematode reproduction.

The number of nematodes in each root system results from a net summation of the number entered, their survival, their progeny, and those that have left the roots. Because of the relatively short term of these experiments and the relatively good condition of the pea roots at their termination (except after scalding) it is likely that migration of nematodes from the roots was slight. Actual proof would require a more exact experimental system.

Assuming that the effects were caused by differences in nematode reproduction, there are at least two different approaches to explaining increased nematode numbers where plant growth was somewhat less

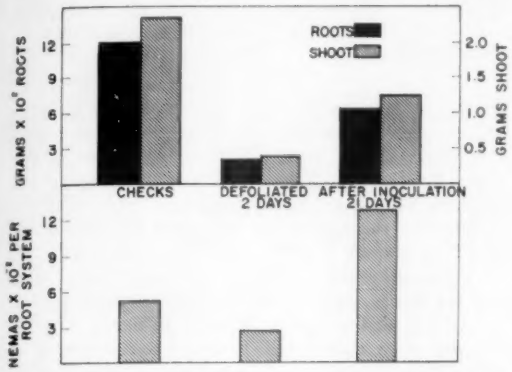


Fig. 6.—Effect of defoliation on numbers of *Pratylenchus penetrans* in root systems and dry weight of Wando peas.

vigorous. One would involve postulation of maximum net synthesis of materials favorable to nematode development and reproduction and/or minimum net synthesis of inhibitory materials under conditions of slightly reduced plant growth. Bird (1) favored a concept of stimulation over one of inhibition. The other would suggest that the plant and the nematode compete for nutrients, the plant under conditions of maximum growth being able to utilize these materials to the detriment of the nematodes. Either explanation at best serves as a hypothesis for additional experimental work.

The treatments imposed on the plants undoubtedly caused changes in the patterns of metabolic products within the plant tissues, and some of these are likely to be correlated with changes in nematode numbers. The important task, however, is to discover the meaningful changes, those that determine plant host suitability for the nematode parasites and/or susceptibility to nematode pathogens. Such studies will require skill, care, and imagination, and an appreciation of the continual interaction of nematode, host and/or suscept plant, and their environment.—Department of Botany, Cornell University, Ithaca, New York.

LITERATURE CITED

1. Bird, A. F. 1960. The effect of some single element deficiencies on the growth of Meloidogyne javanica. Nematologica 5:78-85.

2. Chapman, R. A. 1954. Meadow nematodes associated with failure of spring-sown alfalfa. Phytopathology 44: 542-545.

3. Chapman, R. A. 1958. The effect of root-lesion nematodes on the growth of red clover and alfalfa under greenhouse conditions. Phytopathology 48:525-530.

4. Christie, J. R., and V. G. Perry. 1951. Removing nematodes from soil. Proc. Helminthol. Soc. Wash., D. C. 18:106-108.

5. Collins, J. L., and H. R. Hagan. 1932. Nematode resistance of pineapples. Varietal resistance of pineapple roots to the nematode Heterodera radicicola (Greef) Muller. J. Heredity 23:459-465, 503-511.

6. Courtney, W. D., and H. B. Howell. 1952. Investigations on the bent grass nematode, Anguina agrostis (Steinbuch 1799) Filipjev 1936. Plant Disease Reptr. 36:75-83.

7. Garren, K. H. 1953. Stem nematode disease of greenhouse Hydrangeas. Plant Disease Reptr. 37:340-342.

8. Goto, K., and H. Fukatsu. 1952. Studies on white tip of rice plant caused by Aphelenchoides oryzae Yokoo. II. Number and distribution of the nematode on the affected plants. Ann. Phytopathol. Soc. Japan 16:57.60. (Japanese, English summary.)

9. Hoagland, D. R., and D. I. Arnon. 1938. The water-culture method for growing plants without soil. Univ. Calif. Agr. Expt. Sta. Circ. 347. 39 p.

10. Kirkpatrick, J. D., W. F. Mai, E. G. Fisher, and K. G. Parker. 1959. Population levels of Pratylenchus penetrans and Xiphinema americanum in relation to potassium fertilization of Montmorency sour cherries on Mazzard root stock. (Abstr.) Phytopathology 49:543.

11. Oteifa, B. A. 1951. Effects of potassium nutrition and amount of inoculum on rate of reproduction of Meloidogyne incognita. J. Wash. Acad. Sci. 41:393-395.

12. Oteifa, B. A. 1953. Development of the root-knot nematode, Meloidogyne incognita, as affected by potassium nutrition of the host. Phytopathology 43:171-174.

13. Ritter, M., and R. Ritter. 1958. Influence de l'âge de la plante-hôte sur le développement de Meloidogyne incognita, nematode phytoparasite. Compt. Rend. 246: 2054-2056.

14. Seinhorst, J. W. 1955. Een eenvoudige methode voor het afscheiden van aaltjes uit grond. Tijdschr. Plantenziekten 61:188-190. (English summary.)

15. Sher, S. A., and M. W. Allen. 1953. Revision of the genus Pratylenchus (Nematoda: Tylenchidae). Univ. Calif. Berkeley Publs. Zool. 57:441-469.

16. Snedecor, G. W. 1956. Statistical methods. The Iowa State College Press, Ames. 543 p.

17. Taylor, A. L. 1942. Root-knot resistance of five soybean varieties (Abstr.) Phytopathology 32:650.

TOMATO LEAF COMPOSITION IN RELATION TO BACTERIAL SPOT

M. V. Nayudu and J. C. Walker

Research supported by grants from the Wisconsin Alumni Research Foundation through the Research Committee of the Graduate School, University of Wisconsin, and from the Herman Frasch Foundation. Accepted for publication November 11, 1960.

SUMMARY

The slower development of bacterial spot with age of tomato leaf was correlated with a decrease in total and soluble nitrogen in the leaf tissue. A shift in the balance of amino acids was also probably important in determining pathogenesis. A difference in amino acid balance was also evident between plants grown at the optimum temperature for disease development and plants grown at suboptimum alternating warm-day, cool-night temperatures. Disease development was markedly retarded by change in nutrients supplied the host only when the osmotic value of the nutrient was raised by increasing the levels of KCl, NaCl, or $NaNO_3$. Decrease in disease index was closely correlated, in these cases, with reduced growth of the host, high osmotic value of the nutrient, lower level of Ca, and a higher level of K or Na in the tissue.

We reported earlier (5) that the development of bacterial spot in tomato leaves, incited by *Xanthomonas vesicatoria* (Doidge) Dowson, was influenced by 3 distinct factors: age of leaf at inoculation, air temperature, and nutrition of the host. We also showed (6) that the pathogen on culture medium is incapable of using inorganic salts as a source of nitrogen. Moreover, the luxuriant growth supported by casein hydrolysate was not secured when any one of 17 amino acids was used alone or in various combinations with others, except where all were used in about the same proportions as in the hydrolysate. Amino acid balance was highly important to maximum growth. Herein are the results of a continuation of this study in which was considered the composition of leaf tissue produced under the various conditions known to be favorable or unfavorable to the pathogenic development of the pathogen.

Methods and Materials.—Tomato var. Bonny Best was the test plant. Seedlings were raised in vermiculite until the first true leaf developed. For studies on temperature and age of leaf they were then selected for uniformity and transplanted to compost soil in 4-in. clay pots. For nutrition studies seedlings were transplanted to quartz sand in glazed porcelain crocks provided with a drainage hole covered on the inside with Pyrex glass wool. The nutrients were applied to the sand at intervals of 5–7 days. Except where stated otherwise, plants were grown at 26 ± 2°C under 12-hour day length. Daylight was supplemented by 200-W incandescent lamps.

The isolate was that described previously (5, 6). Inoculum was a 48-hour culture grown on potato-dextrose agar, washed into sterile distilled water, and diluted to give about 75% transmission of light as measured on a Lumetron colorimeter at 620 mμ with a red filter. Except in age-of-leaf experiments, plants were inoculated in the 6-leaf stage by spraying the bacterial suspension onto the lower surface of the leaflets. No moist chamber was used. Degree of disease on a scale of 1–4 has been described (5).

In preparing leaf tissue for analysis, leaflets from selected leaves were cut at the base, quickly weighed, wrapped in cheesecloth, and frozen in liquid air. The samples were transferred to dry ice and stored at −25°C for future use. Freeze-drying and extraction of samples were carried out according to Rohringer et al (8). Samples were prepared and analyzed for amino acids and organic acids by 2-dimensional chromatography as described by Rohringer et al (8). Samples were prepared for analysis of free amino acids on a Beckman amino acid analyzer according to Stein and Moore (9). Total nitrogen was determined by the Kjeldahl method (3). Soluble N was determined by the method described by Umbreit et al (11). Analysis for polyphenols was by the method of Uritani and Muramatsu (12). For determination of inorganic elements, tissue was dried 48 hours at 80°C, and ground in a Wiley mill with a 40-mesh screen; analyses were made with a flame spectrophotometer by H. H. Hull, Soils Department, University of Wisconsin.

Results.—*Age of leaf.*—Table 1 gives total N, soluble N, and polyphenols in leaves from plants grown in July. Samples were taken from the fifth and sixth leaves (numbered from the base of the plant) of inoculated and uninoculated plants at 6, 9, and 13 days after inoculation. Uninoculated leaves were thus passing from extreme susceptibility to moderate resistance, whereas inoculated leaves were passing from an early to a late stage of disease development. There was little difference between inoculated and uninoculated leaves in total and soluble N at each sampling. At 9 and 13 days, phenol content was distinctly higher in infected than in corresponding healthy leaves. In both healthy and infected leaves total and soluble N decreased consistently with age of leaf, while phenols increased consistently.

Table 1.—Nitrogen and polyphenol content of healthy (H) tomato leaves and those infected (I) with bacterial spot at 3 stages of growth and disease development, July, 1958.

Days after	Total N[a]		Soluble N[a]		Polyphenols[a]	
inoculation	H	I	H	I	H	I
6	5.02	4.94	.42	.41	.169	.146
9	4.68	4.42	.39	.41	.362	.691
13	3.29	3.26	.27	.27	.622	.875

[a] Values are percentages of dry weight of tissue.

Table 2.—Relation of nitrogen and polyphenol content of healthy (H) tomato leaves and those infected (I) with bacterial spot at 3 stages of growth and disease development, May, 1959.

Days after inoculation	Upper or lower leaves	Total N[a]		Soluble N[a]		Polyphenols[a]	
		H	I	H	I	H	I
0	Upper	5.54		.43		.017	
	Lower	3.74		.41		.012	
6	Upper	5.54	5.13	.39	.23	.012	.015
	Lower	3.89	3.43	.30	.24	.007	.008
9	Upper	3.48	5.54	.30	.50	.008	.013
	Lower	5.07	4.05	.40	.35	.012	.010
15	Upper	4.20	4.20	.35	.27	.015	.015
	Lower	3.18	3.18	.25	.25	.010	.011

[a] Values are percentages of dry weight of tissue.

A similar experiment was run in early May. Samples of older lower leaves (6–10th from the base) and younger upper leaves (14–18th from the base) were taken from healthy and from inoculated plants at time of inoculation and 6, 9, and 15 days after inoculation. The phenol content was much lower in this series (Table 2) than in the first experiment, and there was little difference between infected and healthy leaves and between samples of leaves of different ages. There was with one exception (lower leaves at 9 days) a decrease in total and soluble N in lower (more resistant) leaves below that in upper (more susceptible) leaves. There was a general trend of reduction in total and soluble N with time of sampling.

The samples taken at inoculation and 15 days after were analyzed for free amino acids, amides, and ammonia on a Beckman amino acid analyzer. Lysine, tyrosine, hydroxylysine, leucine, methionine, and histidine were not detected or occurred in relatively small amounts. The amounts of the other compounds in a single run are recorded in Table 3. Ammonia and glutamic acid in healthy leaves were but little less than in infected upper and lower leaves 15 days after inoculation. The same was true for proline in upper but not lower leaves. Glutamine was distinctly greater in infected upper and lower leaves than in comparable healthy leaves. With all other amino compounds amounts were lower in infected than in healthy leaves. This indicated that the organism used most of the amino compounds in different degrees during pathogenesis. With most compounds there was a decrease in amount in healthy leaves during the 15-day aging period, but there was little or no reduction of glutamic acid, aspartic acid, and phenylalanine in upper leaves. Proline increased during the 15-day period in upper and lower leaves. It seems probable that the reduction in total soluble nitrogen with aging of leaves shown in Table 2 may have been due to lowering of the amounts of some amino compounds and not of others. This would lead to a change in balance of these compounds in the tissue available to the pathogen. In view of the effect of balance of amino acids on growth of the organism *in vitro* (6), it is suggested that the increase in resistance of leaf tissue with aging may be due not only to a decrease in available nitrogenous compounds but also, in part at least, to the proportion of such compounds to one another in the host substrate.

Influence of temperature.—It has been shown (5) that a continuous 24°C was most favorable for disease development, and 16° was least favorable. With a day temperature of 28° and a night temperature of

Table 3.—Relative amounts of free amino acids, amides, and ammonia in upper and lower healthy tomato leaves at time of inoculation and in comparable healthy leaves and those infected with the bacterial spot organism 15 days after inoculation[a]

Compound	Upper leaves			Lower leaves		
		15 days after inoc.			15 days after inoc.	
	At inoc.	Healthy	Infected	At inoc.	Healthy	Infected
Ammonia	2.250	2.060	2.580	2.640	2.100	2.100
Glutamic acid	1.204	1.266	1.030	1.002	.924	1.009
Aspartic acid	.640	.600	.495	.882	.404	.390
Phenylalanine	.092	.096	.062	.082	.060	.039
Proline	.121	.240	.272	.172	.183	.133
Glutamine	.706	.411	.500	.477	.220	.263
Alanine	.867	.423	.355	.678	.264	.208
Serine	.410	.297	.268	.589	.356	.277
Glycine	.355	.267	.247	.445	.202	.188
Valine	.097	.075	.066	.075	.061	.036
Isoleucine	.052	.042	.035	.053	.026	.022

[a] Values are in micromoles per 100 mg of dry tissue.

Table 4.—Relative amounts of certain amino compounds in healthy tomato leaves and in comparable leaves affected with bacterial spot from plants incubated at 24°C continuously and from those kept at 28° day and 16° night.

	Intensity and/or size of spot in[a]			
	Healthy leaves at		Infected leaves at	
Amino acid	24°	28-16°	24°	28-16°
Glutamine	2	1	1	1
Leucine/isoleucine	1	0.5	1	0.5
Valine	1	0.5	1	0.5
Alanine	2	1	2	1
Lysine	0.5	0	0.5	0
γ-aminobutyric acid	3.5	1	4	2
Serine	1	1.5	3	1
Cystine	0.5	0.5	0.5	1
Aspartic acid	4	4	3	4.5
Glutamic acid	4	4.5	2	4
Phenylalanine	0.5	0.5	0.5	0.5

[a] Values are averages of indices from 2 chromatograms. Values for individual spots ranged from 0, for no color, to 6, for very intensely colored and/or large spot on the paper.

16°, however, the disease index was as low as that from a continuous 16°. Leaf tissues were compared from plants continuously at 24° and plants at 28–16° day-night. Leaves were collected from each group from infected and from healthy plants 9 days after inoculation. Since the Beckman amino acid analyzer was not available the tissues were analyzed for amino acids by paper chromatography. Analysis for organic acids was also made by paper chromatography. Citric acid was lowest in both healthy and infected leaves of 28–16° plants, whereas with malic acid the reverse was true. The results with amino compounds are given in Table 4. There was little difference in aspartic acid and glutamic acid in healthy leaves of the 2 temperature treatments, but they were distinctly higher in infected leaves of 28–16° plants where disease progress was slow when compared with infected leaves at 24°. In the cases of leucine/isoleucine, valine, alanine, lysine, and γ-aminobutyric acid the amount in healthy and in infected leaves of 28–16° plants was about one-half of that in 24° plants. These results suggest that changes in the balance of amino compounds as well as organic acids during the incubation period at different temperatures after inoculation may have an influence on growth of the pathogen and consequent disease development although the changes in amino compounds were not always of the same order as in the age-of-leaf experiments.

Influence of nutrition.—It was shown (5) that disease development was severe on the young leaves of plants growing in quartz sand supplied with basal Hoagland's solution containing 200 ppm N, 31 ppm P, 230 ppm K, and having an osmotic value (OV) of 1 atmosphere (atm). There were no significant changes in disease index with variation in concentration of these elements in the balanced solution, or in solutions in which N, P, or K was increased, except when the osmotic value of the solution concerned was at a relatively high level. Thus reductions in disease index were significant only in plants grown on one or the other of the following 3 nutrient solutions: 1) a balanced high OV solution in which OV was raised to 5.7 atm by addition of NaCl; 2) a high-N solution, in which N was increased to 1600 ppm and OV to 5.7 atm by the addition of $NaNO_3$; 3) a high-K solution, in which K was increased to 1600 ppm and OV to 2.4 atm by the addition of KCl. Plants were grown in each of these 3 solutions and in basal Hoagland's solution to the 5-6-leaf stage. At this time 6 plants of each treatment were inoculated. Fourteen days later the disease indices were recorded and fresh weights of tops of 2 healthy plants from each treatment were determined. From 12 or more plants of each treatment the fifth and sixth leaves were collected and prepared for analysis.

Table 5 shows that disease index was highest in basal, intermediate in high-K, and lowest in high-OV and high-N plants. There were correlations between disease index, osmotic value of nutrient solutions, and plant growth. There was little or no difference between treatments in total and soluble nitrogen. When tissue was analyzed for amino acids by paper chromatography there were no differences between treatments in aspartic acid, glutamic acid, phenylalanine, glutamine, leucine, and threonine. There was little difference in glycine and in serine. Alanine and γ-aminobutyric acid were highest in plants grown in basal and high-K solutions. Valine was de-

Table 5.—Relation of nutrient supplied the host to tomato leaf composition and to degree of development of bacterial spot.

				Per cent of dry wt. of element indicated					
Nutrient	Osmotic value of nutrient	Avg. wt. (g) of healthy plants	Disease index[a]	Total N	Sol. N	P	K	Na	Ca
Basal[b]	1	45	79	4.9	.36	.11	3.8	0.4	4.2
High OV[c]	5.7	14	4	5.1	.35	.20	3.9	4.5	2.9
High N[d]	5.7	11	25	4.9	.40	.19	3.9	3.4	2.2
High K[e]	2.4	28	54	4.7	.44	.13	9.4	0.3	1.7

[a] 0 = no signs of disease; 100 = severe disease.
[b] 200 ppm N, 230 ppm K, 31 ppm P, 200 ppm Ca, A-Z solution 1 ml/L.
[c] Similar to [b] except for increase in osmotic value with NaCl.
[d] Similar to [b] except for increase to 1600 ppm N with $NaNO_3$
[e] Similar to [b] except for increase to 1600 ppm K with KCl.

tected only in high-N and high-OV plants, whereas proline occurred in all but the basal-solution plants. It appears that relative amounts of N were not responsible for the wide differences in disease indices of plants grown in the basal and the 3 high-osmotic-value solutions.

When the relative levels of P, K, N, and Ca in plants submitted to the 4 treatments are examined, only Ca is consistently lower in the plants grown in the respective high-osmotic-value solutions than in those in the basal solution. This is expected since it has been observed commonly that high levels of K and/or Na ions in the nutrient or soil solution interfere with absorption of Ca ions. It is also to be noted that plants grown in high-K solution were high in K, whereas high-N and high-OV plants, where excesses of $NaNO_3$ or NaCl were used in the nutrients, showed high levels of Na. The extremely low disease indices in plants in these treatments were correlated with high levels of K or Na in the tissue, with lower Ca levels, and with reduced growth of the plant, and not with any measurable change in nitrogen metabolism.

Discussion.—Nitrogen and polyphenol content of plants have been correlated with susceptibility or resistance to certain diseases. Van Gundy and Walker (13) reported that high amino nitrogen in the leaves of cucumber favored the development of angular leaf spot. In a study of snow blight disease of wheat, incited by *Typhula incarnata* Fr., Tomiyama (10) found that older, more susceptible leaves contained less protein and greater amounts of ammonia nitrogen than younger, more resistant leaves. In a study of blast of rice incited by *Piricularia oryzae* Cav. and leaf spot of rice incited by *Helminthosporium oryzae* Breda de Haan, Wakimoto and Yoshii (14) reported greater polyphenol content of the plant, especially in the leaves, as the plants grew, and in infected as compared with healthy leaves. Leaves with leaf spot showed reduction in soluble and insoluble N, in reducing sugars, and in soluble carbohydrates, and an increase in the insoluble carbohydrates (1).

In the present study young tomato leaves contained greater percentages of total and soluble N than old leaves, and the decrease in N was correlated with decrease in severity of disease. The decrease in total N may have been due to an increase in the activity of proteolytic enzymes, and this might have been enhanced by infection. The reduction of soluble nitrogen in old leaves, healthy or infected, may have been due to translocation to younger leaves, in consequence of which the degree of disease development in older leaves was reduced. The increase in amount of free ammonia in the upper leaves 15 days after inoculation suggests increased activity of deaminases. Similar increase in ammonia was reported in oak leaves infected with *Microsphaera alphitoides* Griff. & Maubl. (4), and in tomato crown gall incited by *Agrobacterium tumefaciens* (E. F. Sm. & Towns.) Conn. (7). Decrease in the quantity of some amino acids and an increase in some others with leaf maturity may be due to transamination reactions leading eventually to changes in the proportion of amino acids to one another.

The lower phenolic content of leaves in the spring than in the midsummer experiment, and the little difference in the former case between old, resistant leaves and young, susceptible ones, indicate that phenols were not necessarily related to resistance. On the other hand, since the balance of amino acids was shown to be important in determining growth of the pathogen *in vitro* (6), it appears that the shift in relative amounts of amino acids in old as compared with young leaves might be responsible in part for the change in the relative resistance of old tissue.

The influence of temperature on the development of bacterial spot of tomato appears related to relative quantities of the amino acids in the leaf tissue. An increase in malic acid under low night-temperature conditions suggests that carbohydrate metabolism is also affected. Preliminary work on sugars by paper chromatography indicated no significant changes in healthy and infected samples. In winter-grown plants very little free sugar was detectable. Van Gundy and Walker (13) reported a higher content of soluble carbohydrates in young leaves than in old leaves of cucumber, and fluctuations in carbohydrate content at any time of the day were not sufficient to inhibit growth of the angular leaf spot organism (*Pseudomonas lachrymans* (E. F. Sm. & Bryan) Carsner) as evidenced by its requirements in culture. With the more conspicuous changes noted in the nitrogenous constituents of the plants grown under various conditions, and because of the importance of carbohydrates in protein synthesis rather than in proteolysis, extensive investigation of the carbohydrates was not undertaken in this study.

A high level of K in the nutrient resulted in accumulation of potassium, but high N supply had no significant effect on nitrogen accumulation (Table 5). Headley et al (2), working with wheat seedlings, reported that sand had less absorptive capacity for Na salts than soil and that the green weight of plants in sand was 62% of that of plants grown in loam soil. Also, only 15% of applied NaCl was absorbed by the loam soil, as compared with 77% of Na_2CO_3, 85% of $NaHCO_3$, and 53% of Na_2SO_4. This suggests that the large reduction in growth of high N plants and of plants grown on a balanced solution of high osmotic value might have been due in part to the properties of the sand used to grow the plants and partly to the type of sodium salts used. It should be emphasized that the tomato plant shows selective absorption of elements and that low Ca under abnormal nutritional conditions is more than likely due to the antagonism of K ions to Ca ions.

A much more extensive analysis of leaf tissue is necessary to determine all the factors that may influence rate of disease development. The data presented indicate that changes in the proportions of amino

acids and amides are important in influencing pathogenesis by the bacterial spot organism.

LITERATURE CITED

1. Asada, Y. 1957. Studies on the susceptibility of akiochi (autumn-decline) rice plant to Helminthosporium blight. III. Changes of nitrogen compounds, carbohydrates, reducing ascorbic acid and respiration accompanied by the infection of Cochliobolus miyabeanus and existence of hyphae in diseased spots. (Trans. title) Ann. Phytopathol. Soc. Japan 22:103-106. (Japanese, with English summary.)
2. Headley, F. B., E. W. Curtis, and C. S. Scofield. 1916. Effect on plant growth of sodium salts in the soil. J. Agr. Research 6:857-869.
3. Hiller, Alma, A. J. Plazin, and D. D. Van Slyke. 1948. A study of conditions for Kjeldahl determination of nitrogen in proteins. J. Biol. Chem. 176:1401-1420.
4. Kiraly, Z., and G. L. Farkas. 1959. Biochemical trends in plant pathology. Phytopathol. Z. 34:341-364.
5. Nayudu, M. V., and J. C. Walker. 1960. Bacterial spot of tomato as influenced by temperature and by age and nutrition of the host. Phytopathology 50:360-364.
6. Nayudu, M. V., and J. C. Walker. 1961. In vitro nutrition of the tomato bacterial spot organism. Phytopathology 51:32-34.
7. Riker, A. J. 1939. Physiological relations between host and parasite in crown gall—an example of basic biological research with plant materials. Am. J. Botany 26: 159-162.
8. Rohringer, R., M. A. Stahmann, and J. C. Walker. 1958. Biochemical changes in plant disease: Effect of Fusarium oxysporum f. lycopersici and its metabolites on leaf constituents of susceptible and resistant tomatoes. Agr. and Food Chem. 6:838-843.
9. Stein, W. H., and S. Moore. 1954. The free amino acids of human blood plasma. J. Biol. Chem. 211:915-926.
10. Tomiyama, K. 1955. Studies on the snow blight disease of winter cereals. Rept. Hokkaido Natl. Agr. Expt. Sta. 47:1-234.
11. Umbreit, W. W., R. H. Burris, and J. F. Stauffer. 1957. Manometric techniques. 3rd ed. Burgess Pub. Co. Minneapolis, Minn. 338 p.
12. Uritani, I., and K. Muramatsu. 1952. Studies on the phytopathological chemistry of black-rotten sweet potato. 2. Some factors concerning the color changes of the injured part. (Trans. title) J. Agr. Chem. Soc. Japan 26:289-295. (Japanese, with English summary.)
13. Van Gundy, S. D., and J. C. Walker. 1957. Relation of temperature and host nutrition to angular leaf spot of cucumber. Phytopathology 47:615-619.
14. Wakimoto, S., and H. Yoshii. 1958. Relation between polyphenols contained in plants and phytopathogenic fungi. 1. Polyphenols contained in rice plants. Ann Phytopathol. Soc. Japan 23:79-84. (Japanese, with English summary.)

HOST RANGES AND PROPERTIES OF SOME VIRUSES FROM DAHLIA

R. A. Mildner and R. P. Scheffer

Respectively, former graduate assistant, Michigan State University, now graduate assistant, University of Wisconsin; and Associate Professor, Department of Botany and Plant Pathology, Michigan State University.

Accepted for publication Nov. 14, 1960. Michigan Agricultural Experiment Station Journal Article No. 2600. Photographs were taken by P. G. Coleman.

SUMMARY

Sixteen plant species were tested as hosts for 5 virus isolates from dahlias with leaves that showed ringspots. Two isolates caused ringspots on dahlia seedlings; the others caused non-distinctive symptoms. Thermal inactivation points ranged from 40° to 90°C; dilution end points from 1:10 to 1:1200; resistance to aging *in vitro* from 8 to 480 hours; and resistance to aging in drying leaves from 48 to 720 hours. Two of the isolates were considered to be strains of tomato spotted wilt virus, while the other isolates were not identified with known viruses. One of the unknowns caused ringspots on dahlia leaves.

Standard varieties of *Dahlia pinnata* Cav. generally carry viruses, a situation probably associated with continuous vegetative propagation. Several viruses are involved, but with the exception of mosaic (2) and ringspot caused by tomato spotted wilt virus (TSWV), little is known about these diseases. Viruses other than TSWV also may cause ringspots in dahlia (7). Therefore, 5 virus isolates from plants showing typical ringspots were selected and studied to determine their relationships. Viruses or strains were soon found which apparently are related to TSWV; one of these caused ringspot while another did not. Other isolates, including one which caused ringspot, appeared not to be related to TSWV. Some of the data included herein were published as an abstract (5).

Materials and methods.—Dahlia plants showing ringspots in the field were brought into the greenhouse and grown as virus sources. Such material, collected in fields of commercial dahlias in Michigan, served as the original source of all viruses except isolate 1, which was supplied in dahlia leaves by Dr. R. D. Raabe of the University of California, Berkeley. Plants showing mosaic or other virus-like symptoms were avoided.

Transfers were made from dahlia to *D. stramonium* var. *tatula* L., *Vigna sinensis* Endl., or *Gomphrena globosa* L., using Yarwood's (8) leaf disk method. Single lesions were then removed, after the method of Jensen (3), buffer and Carborundum dust were added, and the mixture was ground on a glass slide with a glass spatula. This preparation was used as inoculum, and the procedure was repeated 4 times in succession. Isolates thus obtained were kept in stock plants and were used in all experiments. The original transfers from dahlia obviously contained mixtures of viruses, because host ranges and symptoms were changed after making the series of single lesion transfers (4) (Fig. 1, A and B).

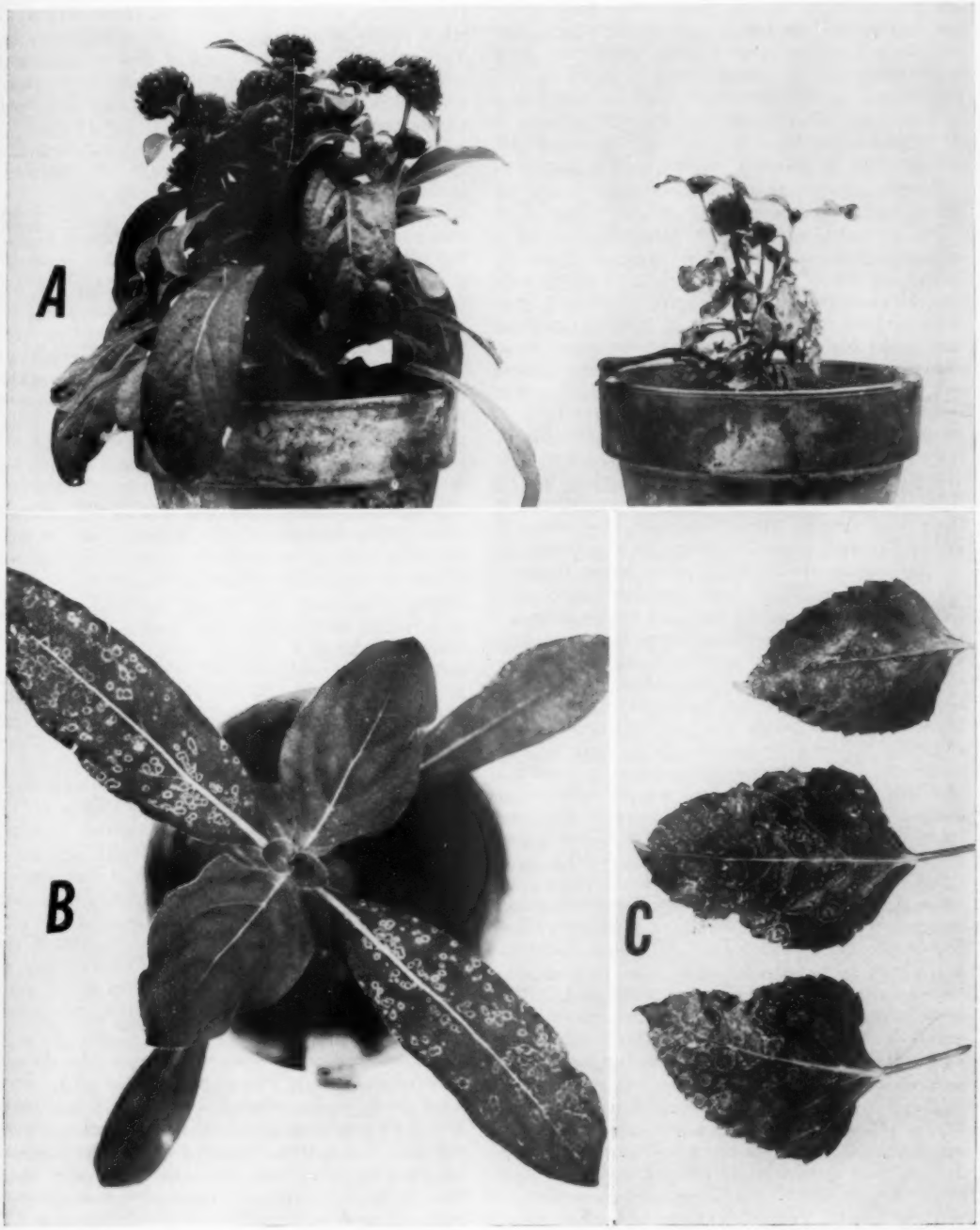


Fig. 1.—Symptoms caused by dahlia virus isolates. **A)** Left, non-inoculated Gomphrena plant; right, symptoms caused by isolate 2 before using single lesion transfers. **B)** Symptoms on Gomphrena caused by isolate 2 after single lesion transfers. **C)** Symptoms on field-grown dahlia leaves which were the source of isolate 3.

Datura and Gomphrena gave countable lesions and were used to determine physical properties. Five plants were inoculated with each sample to compensate for variations in host plant resistance. Leaves just short of full expansion were dusted with Carborundum and rubbed with a glass spatula or index finger which was previously dipped in infectious sap. For thermal inactivation tests, 0.5 ml sap was placed in thin-walled glass tubes, and after 10 minutes at the desired temperature, the tubes were cooled quickly and sap was immediately rubbed on test plants.

Results.—*Symptoms on dahlias.*—Isolate 1 was obtained from dahlia leaves with necrotic rings, about 1 cm in diameter, around a green center. These symptoms apparently were the same as those described by Smith (6) and Brierley (2). Symptoms on dahlia variety Betty Zane, source of isolate 2, were occasional target spots 6–8 mm in diameter, irregular dark green islands and yellow chlorotic spots. Stems and petioles were distorted and some vein banding was evident. The variety Sherwood's Peach, source of isolate 3 (Fig. 1C), had symptoms similar to those of isolate 1. The variety Nicky K, source of isolate 5, had indistinct reddish target spots 4 to 5 mm in diameter. Isolate 6 came from the growing tip of a *Petunia hybrida* Vilm. plant which developed systemic vein clearing after inoculation with isolate 5. Isolate 5 usually caused local lesions in petunia.

After making the series of single lesion transfers, Unwin dahlia seedlings were inoculated with all 5 isolates. Isolate 1 caused indistinct yellow spots 2–3 mm in diameter on the rubbed leaves in 6 to 11 days. Later, some spots became necrotic while others developed target rings. Necrotic areas appeared on stems and lower leaves and many plants were killed. Isolate 2 caused yellow or dark green mottle, occasionally followed by necrotic areas; there were no ringspots. Later, petioles and stems were distorted, leaf tips and margins became necrotic and yellow mottled areas developed in the leaves. All lower leaves were finally killed. Isolate 3 caused yellow spots 2–3 mm in diameter 10 to 15 days after inoculation. Target rings usually developed around the spots. Later, hieroglyphic-like lines developed around the targets or veins, and ringspots appeared on new leaves. Isolate 5 caused pale leaf spots which had a tendency to form wide single rings (not targets). Occasionally, dark green areas were seen. These were followed by large brown necrotic or chlorotic areas. Later, petioles became necrotic, and leaves died. Isolate 6 caused non-distinctive light green spots 2–3 mm in diameter on the inoculated leaves, followed by an indistinct dark green mottle. Leaves turned yellow, making spots very conspicuous. Later, small scattered necrotic spots appeared on young leaves; these spots enlarged and coalesced, and leaves died. After a time no further symptoms were formed on new growth, and infected plants became normal in appearance.

Only isolates 1 and 3 caused ringspots similar to those on the source plants. Change in symptom pattern after single lesion transfers is evidence that mixtures of viruses were present in the source plants.

Some factors affecting transmission.—Sap inoculations from dahlia to dahlia and from dahlia to other plants were unsuccessful but transmission by Yarwood's (8) leaf disk method was effective and reliable. Once transfer was made to hosts other than dahlia, inoculations to determine host range and physical properties were made by expressed sap. Infected leaf tissue was ground in a phosphate buffer solution containing a small amount of 400 mesh Carborundum. A 0.05 *M* buffer solution was optimum for maximum numbers of lesions. The optimum pH was found to vary somewhat from isolate to isolate, but pH 8.5 was uniformly favorable.

Datura gave the best countable lesions for isolates 1, 3, 5, and 6, while Gomphrena was most suitable for isolate 2. These plants were used as both donor and acceptor hosts in assays. First symptoms appeared 3 to 5 days after inoculation. Inoculations were uniformly successful when plants were soft and succulent, well fertilized, grown at a minimum night temperature of 22°C, and under reduced light intensity. Hard, slow-growing plants often failed to show symptoms.

When infected leaf tissue was ground with distilled water rather than with buffer, isolates 2 and 6 were highly infectious, isolate 5 was rarely infectious and isolates 1 and 3 lost infectivity completely.

Host range.—In host range experiments, plants to be tested were usually inoculated in groups of 5; several groups were then inoculated over a period of several days or weeks. Plants of 16 species belonging to 8 families were used (Table 1), and potential hosts were tested many times if symptoms did not appear. The viruses were transferred from all plants which developed symptoms back to the original host for comparison. Usually symptomless plants were not examined for the presence of latent viruses. Differences in host ranges of the 5 isolates soon become apparent (Table 1). Host ranges and symptom types indicated that isolates 1 and 5 were similar, but not identical. Other isolates differed from each other and from isolates 1 and 5, suggesting that they may be different viruses rather than strains of one virus. However, the host range differences may be misleading, since they are based only on visual symptoms. Isolate 6 clearly differed from the others (Table 1).

Physical properties.—Sap was prepared for thermal inactivation tests in two ways. Isolates 1, 3, and 5 were prepared by grinding 1 g infected leaf tissue with 4 ml potassium phosphate buffer solution (.05 *M*, pH 8.5) in a mortar. Isolates 2 and 6 were prepared by freezing tissue and then extracting sap; liquid thus collected was used without further dilution. Isolate 3 was inactivated at a lower temperature (40-42°C) than were the other isolates. Isolate 6 differed markedly from the others, requiring 96°C for inactivation (Table 2).

Table 1.—Hosts of dahlia viruses after purification by single lesion transfers.

Host plants	Isolate no.				
	1	2	3	5	6
Calendula officinalis var. Ball Gold	3/20[a]	5/15	0/25	5/15	0/25
Capsicum frutescens	13/15	3/36	7/18	18/18	0/25
Chenopodium amaranticolor	0/25	16/16[b]	0/25	0/25	0/25
Cucumis sativus var. National Pickling	0/29	0/20	0/25	2/28[b]	25/25[c]
Dahlia pinnata (Seedlings)	31/65	18/75	36/125	7/25	10/90
Datura stramonium var. *tatula*	100/100	4/16	70/100	100/100	100/100[d]
Gomphrena globosa	4/15	100/100	7/15	9/15	15/15
Lycopersicon esculentum var. Bonny Best	18/30	0/42	12/25	15/20	0/65
Nicotiana glutinosa	8/17[b]	3/26	0/20	5/11[b]	10/10[b]
N. tabacum var. Havana-38	6/20[b]	9/17	0/25	10/15	15/15
Petunia hybrida var. Minstrel	22/25[b]	6/14	2/20	15/15[b]	6/12
Phytolacca americana	0/26	16/16[b]	0/25	0/25	0/25
Sinningia speciosa	6/17[b]	14/15	0/33	0/25	0/25
Verbescina encelioides	0/25	1/25	0/25	1/25	0/25
Vigna sinensis var. Black Hog	8/28[b]	28/29	2/18[b]	7/17[b]	0/20
Zinnia elegans var. Brightness	0/26	2/15	0/25	7/15	5/25

[a] Number of plants with symptoms/number of plants inoculated.
[b] Systemic symptoms were absent. There was no attempt to recover the virus from symptomless parts.
[c] Symptomless carrier.
[d] Infection was restricted to local lesions.

Isolates 1, 3, and 5 were prepared for dilution experiments by grinding 1 g infected leaf tissue in 9 ml 0.05 *M* potassium phosphate buffer solution (pH 8.5). This preparation was considered a 1:10 dilution and further dilutions were made with buffer solution. Isolates 2 and 6 were usually prepared by the method described for thermal inactivation, using distilled water for further dilutions. Isolate 3 was infective at a 1:10 dilution, but not at a 1:20 dilution. Isolate 6 was the most tolerant to dilution, being infective at 1:1200 but not at 1:1400. Other isolates fell between these extremes (Table 2). Isolates 3 and 6 therefore differed from each other and from the other isolates.

Sap was prepared as described for thermal inactivation for aging *in vitro* determinations. Sap samples were held in test tubes at 22°C and were removed at intervals and rubbed on test plants. Aging *in vitro* data (Table 2) indicate differences in some of the isolates. Isolates 1, 3, and 5 were equally unstable, being infectious at 6 to 8 hours but not after 12 to 16 hours. Isolate 2 was somewhat more stable, while isolate 6 remained in crude sap much longer than the other isolates and was still active at 480 hours. The data for isolate 2 were not strictly comparable to the data for other isolates, because Gomphrena was the donor plant for isolate 2, and Datura was used for other isolates.

The ability of the viruses to remain active in dry leaves was tested by placing 1 g samples of infected leaves between paper towels. At intervals, a sample was removed, ground in buffer, and rubbed on 5 test plants. Results again indicated differences between the isolates. The most stable isolate was 6, which was infectious at 720 hours. Isolates 1 and 5 were infectious at 96 hours but not at 120 hours, while isolates 2 and 3 were infectious at 48 hours but not at 72 hours. The relationship held in 4 experiments.

When infected leaf tissue was frozen, thawed, and assayed for infectivity, only isolates 2 and 6 remained active. Repeated trials produced an occasional infected plant with isolate 5, but isolates 1 and 3 were always non-infectious after freezing. To determine ability of isolates to survive quick drying and storage, infected leaf strips were dried at 0–4°C under partial vacuum in desiccators containing calcium chloride. Leaf pieces were then pulverized and placed in small vials under the same conditions. Samples were removed at intervals and assayed for infectivity. Isolates 2, 5, and 6 were infectious after 30 days at low temperatures, but isolate 3 lost infectivity. An occasional assay plant inoculated with isolate 1 developed symptoms after 30 days storage at low temperature.

Table 2.—Thermal inactivation, dilution effects, and aging *in vitro* effects on infectivity of dahlia virus isolates. Each value was determined from 4 sets of experiments, using 5 plants per isolate per category in each experiment.

Isolate[a]	Thermal inactivation (°C)	Dilution end point[c] (reciprocals)	Aging end point (hrs.)
1	46/48[b]	100/150[d]	6/8[e]
2	48/50	200/300	32/40
3	40/42	10/20	8/12
5	46/48	200/250	8/12
6	90/96	1200/1400	480/600

[a] *D. stramonium* was used for isolates 1, 3, 5, and 6; *G. globosa* was used for isolate 2.
[b] Infectious after 10 min. exposure to 46°C, but not infectious after 10 min. at 48°C.
[c] Diluent was 0.05 *M* (pH 8.5) potassium phosphate buffer. Isolate 2 was also diluted with distilled water, whereby dilution end point was 60/64.
[d] Infectious when diluted 1:100, but not infectious at 1:150 dilution.
[e] Sap was infectious 6 hours after extraction, but not infectious at 8 hours.

Cross-protection.—The relationship of isolate 6 to the other isolates was examined by cross-protection experiments. Five test plants were inoculated with each isolate. Later, leaves showing strong systemic symptoms were inoculated with isolate 6. In every case Datura and Gomphrena infected with isolates 1, 2, 3, and 5 were not protected against later infection by isolate 6. These results indicate that isolate 6 is not related to the other isolates. The experiment was repeated with the same results.

Discussion.—Physical property and host range data show that several viruses were included in the 5 isolates. Thus isolate 6 clearly differed from all others. Isolates 1 and 5 were similar to each other in spite of minor differences in symptom expression on several hosts. The data for isolates 2 and 3 are less clear-cut; apparently they differ from each other and from the other isolates in physical properties, hosts, and in types of symptoms caused.

Of primary concern was the comparison of the isolates with viruses previously described in the literature. The most obvious of these is tomato spotted wilt virus (TSWV) (1), which is known to cause ringspot on dahlias. Isolates 1 and 3 caused ringspots, but they differed somewhat from TSWV in other respects (4, 5). The characteristic bronzing of tomato caused by TSWV was not seen on tomatoes infected with any of the isolates. TSWV in *N. glutinosa* is systemic and usually kills the plant; isolate 1 caused only local lesions, while isolate 3 had no visible effect. Isolates 1 and 3 did not visibly affect zinnia, but this plant is easily infected with TSWV. Petunia is a good host for TSWV, but the virus is seldom systemic; the plant was difficult to inoculate with isolate 3, but systemic invasion was the rule. Host ranges and properties show that isolate 1 is more like TSWV (1) than is isolate 3. Isolate 5 apparently is related to isolate 1, the most important difference being that it caused mottle rather than ringspot in dahlia seedlings. Thus in spite of minor differences, isolates 1 and 5 are considered to be strains of TSWV. Isolate 6 clearly differs from TSWV in host ranges and physical properties, and isolates 2 and 3 probably differ also.

Host ranges, symptomatology, and physical properties seem to exclude the possibility that any of the isolates were tobacco streak, tomato ringspot, cucumber mosaic, dahlia mosaic, or potato viruses A or Y (7). In Brierley's original work with dahlia ringspot (2) the causal virus was not transmitted mechanically, and no properties were given. However, since isolate 3 caused ringspot but apparently differed from TSWV and other known viruses, Brierley's designation should be retained.—Department of Botany and Plant Pathology, Michigan State University, East Lansing.

LITERATURE CITED

1. Bald, J. G. and G. Samuel. 1931. Investigations on spotted wilt of tomatoes II. Austr. Counc. Sci. & Ind. Res. Bull. 54.

2. Brierley, P. 1933. Studies on mosaic and related diseases of dahlia. Contrib. Boyce Thompson Inst. 5: 235-288.

3. Jensen, J. H. 1933. Isolation of yellow mosaic viruses from plants infected with tobacco mosaic. Phytopathology 23:964-974.

4. Mildner, R. A. 1960. Ringspot and associated virus diseases of dahlias. M.S. Thesis, Department of Botany and Plant Pathology, Michigan State University.

5. Mildner, R. A. and R. P. Scheffer. 1959. Mechanical transmission and host ranges of dahlia ringspot viruses. Phytopathology 49:546 (Abs.)

6. Smith, K. M. 1932. Further experiments with a ringspot virus: its identification with spotted wilt of tomato. Ann. Appl. Biol. 19:305-330.

7. Smith, K. M. 1957. A textbook of plant virus diseases. Little, Brown, and Company, Boston, Mass. 652 p.

8. Yarwood, C. E. 1953. Quick virus inoculation by rubbing with fresh leaf disks. Plant Disease Reptr. 37: 501-502.

RELATION OF SUGAR TRENDS AND PITH DENSITY TO DIPLODIA STALK ROT IN DENT CORN

Jeweus Craig and A. L. Hooker

Respectively, Research Associate and Associate Professor of Plant Pathology, University of Illinois, Urbana. Accepted for publication November 14, 1960. Portion of a Ph.D. thesis, University of Illinois.

SUMMARY

Twelve inbred lines of dent corn were tested by artificial inoculation for reaction to Diplodia stalk rot. Sugar analyses and determinations of pith density were made of stalk samples at intervals during the growing season from uninoculated plants of the inbreds. A numerical scoring system was devised for expressing trends in sugar content during the observation period. Increases in sucrose, reducing sugar, and total sugar, and high pith density were associated with resistance to stalk rot. It is theorized that a decrease in the sugar level of the stalk causes senescense of pith tissue, indicated by a decrease in pith density, and plants with senescent pith tissue are susceptible to stalk rot.

Several investigators (4, 6, 9) have reported that resistance to stalk rot of corn caused by *Diplodia zeae* (Schw.) Lev. or *Gibberella zeae* (Schw.) Petch is associated with high carbohydrate levels in the stalk, but these reports present little critical experimental evidence to substantiate this theory.

Pappelis (10, 11) correlated resistance to stalk rot from *D. zeae* with the density of pith tissue in

the stalk. Pith tissue of high density had a high percentage of living cells, whereas dry fluffy pith of low density was composed mostly of dead cells. He suggested that the progress of the pathogen was inhibited by phenolic compounds formed when living cells were invaded. Wernham (12) has since reported a positive correlation between susceptibility to stalk rot caused by *G. zeae* and the percentage of dry pith in the stalk internode.

This paper reports experimental evidence for a relationship of sugar content of the stalk and of senescence of pith tissue to susceptibility to stalk rot caused by *D. zeae*.

Materials and methods.—Twelve inbred lines of dent corn (38-11, B14, C103, C. I. 4-8, R4, R53, R80, R101, R109B, R130, R168, and WF9) were selected for study. In previous tests these inbreds had differed widely in their reaction to stalk rot. These tests employed a diversity of Diplodia isolates and were conducted at different locations for several years. Stalk rot reaction of each inbred was consistent. Plantings were made May 19, 1959, on the Agronomy South Farm of the University of Illinois. The inbreds were planted in a split-plot experimental design using 4 replications. The main plots of each replicate were 10 hills of each inbred planted in random order, the plants thinned to 4 per hill. The plants from the first 2 hills of each main plot and alternate hills thereafter were used to provide samples for sugar analyses and pith-density determinations. The plants in the remaining hills were inoculated with *D. zeae* to determine the reaction of the inbred lines to stalk rot.

One week after silking, the plants were inoculated by injecting a water suspension of conidia of *D. zeae* into the second internode above the ground (7). A single isolate, of proven pathogenicity, obtained from corn in Illinois was used. Development of rot, as indicated by discolored tissue, was observed at weekly intervals for 4 weeks, beginning 1 week after inoculation. The stalks were cut lengthwise and scored for stalk rot on the following scale: 1) 25% or less of the inoculated internode rotted, 2) 26-50% of the inoculated internode rotted, 3) 51-75% of the inoculated internode rotted, 4) 76-100% of the inoculated internode rotted, 5) rot present in the internodes adjacent to the inoculated internode, and 6) plant prematurely killed.

The stalk samples used for sugar analyses and pith-density determinations were from uninoculated plants showing no evidence of natural stalk rot infection and normal in other respects. The first sample from each of the 12 inbreds was collected 8 weeks after planting. Subsequent sampling dates, based on the maturity stages of the inbred lines, were taken at silking and 2, 3, and 4 weeks after silking. For each inbred the collection dates of the last 3 samples coincided with the dates of rot observations in inoculated plots. In all cases samples and rot observations made the same date were taken from adjacent hills.

Sections from the second internode above the ground of all stalks in a hill were bulked to form each sample. Samples were taken in each of the 4 replicates between 6 and 7 a.m. Central Standard Time. Immediately after cutting, they were placed in small metal containers and covered with crushed ice. The samples were placed in a freezing compartment within 45 minutes of collection and held at −5°C until processed (usually within 8 hours of collection).

The rind and nodal tissues were removed from the stalk sections, and all future studies were made on the remaining pith tissue. Throughout the paper the terms "rind" and "pith" are used to indicate general regions of the corn stem. "Rind" tissue is the exterior portion of the stem, composed of the epidermis, sclerified parenchyma, and closely grouped vascular bundles. "Pith" tissue is the soft spongy tissue in the interior of the stem composed of thin-walled parenchyma cells and widely scattered vascular bundles. Determinations of pith density were made at each of the last 4 sampling dates by the method described by Pappelis (10). Cylinders of tissue ¼ in. in diameter were cut with a cork borer from the centers of the internodal sections. These pith cores were weighed and the volume determined by water displacement. Specific gravity of the central pith tissue was calculated from these measurements.

Following the density determinations, the pith cores and remnants of samples were dried in an evacuating oven for 36 hours at 75°C. The dried samples were ground in a Wiley mill fitted with a 1-mm screen and stored in sealed paper bags at room temperature until analyzed. The samples were analyzed for sugars by the Munson-Walker method (1). HCl was used for the inversion of sucrose. The results are reported as dextrose equivalent on a moisture-free basis as the relationship of sugar concentration to stalk rot reaction was similar when sugar percentages were expressed either on a dry-weight or fresh-weight basis (3).

The data obtained from the observations of stalk rot, sugar analyses, and determinations of pith density were analyzed statistically, and significant differences were determined with Duncan's multiple range test (5).

Results.—*Stalk rot reaction.*—The 12 inbred lines differed markedly in reaction to stalk rot (Table 1). The inbred lines, on the basis of rot score at the final observation, were classed as resistant, intermediate, or susceptible (Table 2). Those classed as resistant formed a statistically distinct group, but separation of the remaining lines into intermediate and susceptible groups was in some cases arbitrary because of intergradation in the ranking of the rot scores.

Sugar analyses.—Differences were great among the 12 inbred lines in percentages of sucrose and of reducing sugars (Tables 3 and 4). A comparison of Table 1 with Tables 3 and 4, however, shows no consistent relation between rot score and sugar content until the fourth week after silking. At that time the

Table 1.—Stalk rot scores of 12 inbred lines of dent corn over a 4-week period following inoculation.

Week	Rank at week indicated, and mean rot scores[a]											
1	WF9 (1.0)	C103 (1.0)	R101 (1.0)	R4 (1.1)	B14 (1.2)	C.I. 4-8 (1.2)	R130 (1.3)	38-11 (1.3)	R80 (1.6)	R109B (1.7)	R168 (2.3)	R53 (3.8)
2	C103 (1.0)	R4 (1.0)	B14 (1.1)	WF9 (1.2)	R130 (1.3)	R101 (1.3)	C.I. 4-8 (2.2)	R168 (3.3)	R109B (3.5)	38-11 (3.7)	R80 (4.0)	R53 (4.0)
3	C103 (1.0)	R4 (1.0)	WF9 (1.1)	B14 (1.3)	R130 (1.3)	R101 (1.4)	C.I. 4-8 (2.7)	R109B (3.4)	R168 (3.9)	R53 (4.0)	38-11 (4.0)	R80 (4.8)
4	C103 (1.2)	B14 (1.3)	R101 (1.6)	WF9 (1.9)	R130 (3.0)	C.I. 4-8 (3.4)	R109B (3.8)	38-11 (3.8)	R53 (4.3)	R4 (4.4)	R168 (5.1)	R80 (5.6)

[a] Mean rot scores are the average of 4 replications and are indicated in ( ).
Means not underscored by the same line are significantly different at the 5% level.

most susceptible lines were among the lowest of the inbreds in per cent of sucrose and reducing sugars. On a basis of sugar content, however, the resistant inbreds could not be differentiated from the intermediate inbreds.

Changes in sugar levels during the observation period appeared to be related to stalk rot rankings (Fig. 1, 2, 3). Susceptible inbred lines exhibited a downward trend in content of reducing sugar after silking. These inbreds also showed a decrease in sucrose at the end of the observation period, but the decrease in reducing sugar content occurred sooner after silking and seemed more consistent.

Inbred lines classed as intermediate, except for R130, maintained the level of sucrose and reducing sugars throughout the observation period. R130 showed a decrease in reducing sugar content after pollination, and an increase in sucrose.

The resistant inbreds, except for WF9, were characterized by increases in sucrose content, whereas the content of reducing sugars remained relatively stable. WF9 exhibited an upward trend in reducing sugars after silking, and no marked change in sucrose level.

A sugar-trend scoring system was devised that provided a numerical expression of significant in-

Table 2.—Stalk rot reaction classes and rot scores of 12 inbred lines of dent corn 4 weeks after artificial inoculation with *Diplodia zeae*.

Resistant		Intermediate		Susceptible	
Inbreds	Score	Inbreds	Score	Inbreds	Score
C103	1.2	R130	3.0	R53	4.3
B14	1.3	C.I. 4-8	3.4	R4	4.4
R101	1.6	R109B	3.8	R168	5.1
WF9	1.9	38-11	3.8	R80	5.6

Table 3.—Percentage of sucrose on a dry-weight basis in stalk samples of 12 inbred lines at various intervals after planting.

Sampling date	Rank of inbreds and mean per cent of sucrose[a]											
8 weeks after planting[b]	R168 (9.3)	R53 (8.7)	C.I. 4-8 (6.7)	R130 (6.0)	WF9 (5.7)	R80 (5.7)	B14 (5.3)	38-11 (4.7)	R109B (4.5)	R101 (4.0)	C103 (2.5)	R4 (1.9)
At silking	C.I. 4-8 (27.0)	R168 (23.7)	WF9 (18.0)	R4 (15.5)	C103 (14.3)	R109B (11.3)	R53 (8.7)	B14 (6.3)	38-11 (6.0)	R101 (4.5)	R80 (4.3)	R130 (4.3)
2 weeks after silking	C.I. 4-8 (32.0)	C103 (28.0)	R130 (27.5)	WF9 (24.3)	R109B (23.3)	R168 (23.3)	R53 (22.5)	R4 (19.0)	B14 (13.5)	R80 (11.3)	38-11 (9.70)	R101 (8.0)
3 weeks after silking	C.I. 4-8 (46.5)	C103 (38.5)	R130 (28.5)	R53 (24.7)	R109B (23.7)	WF9 (23.3)	R168 (21.0)	R101 (20.3)	R4 (19.7)	R80 (14.3)	38-11 (13.3)	B14 (12.7)
4 weeks after silking	R130 (38.7)	R109B (38.3)	C103 (28.7)	C.I. 4-8 (26.0)	B14 (24.5)	WF9 (22.5)	38-11 (13.5)	R101 (13.0)	R4 (11.3)	R53 (9.3)	R80 (8.5)	R168 (8.5)

[a] The inbred means for sugar percentage indicated in ( ) are the average of 4 replications, arranged in order of rank. Means not underscored by the same line are significantly different at the 5% level.
[b] These means were not analyzed for significant differences, because of variation in maturity of the inbred lines.

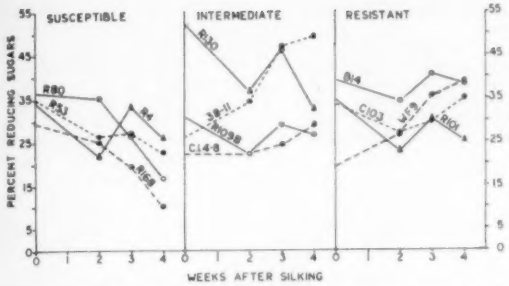


Fig. 1.—Percentage of reducing sugars on a dry-weight basis in stalks of 12 inbred lines during a 4-week period following silking. Each observation is the mean of 4 replications.

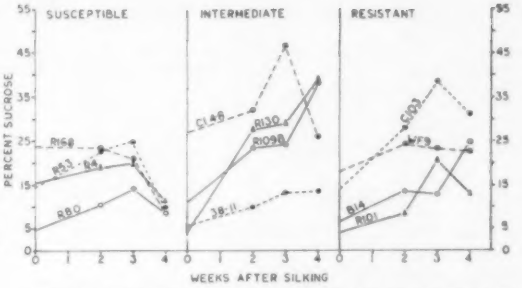


Fig. 2.—Percentage of sucrose on a dry-weight basis in stalks of 12 inbred lines during a 4-week period following silking. Each observation is the mean of 4 replications.

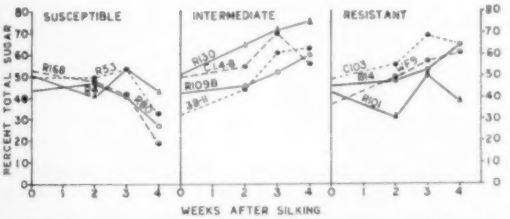


Fig. 3.—Percentage of total sugars (sucrose and reducing) on a dry-weight basis in stalks of 12 inbred lines during a 4-week period following silking. Each observation is the mean of 4 replications.

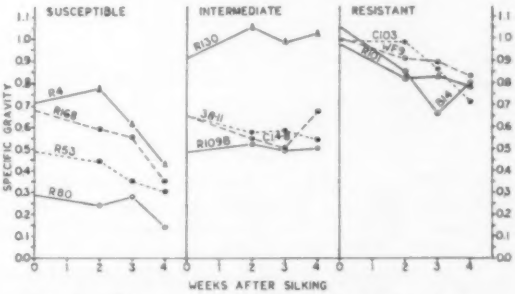


Fig. 4.—Pith-core density of 12 inbred lines during a 4-week period following silking. Each observation is the mean of 4 replications.

creases or decreases in sugar level during the observation period. Each inbred was assigned a value of +4 for the percentage of sucrose and reducing sugars present at silking. Any statistically significant (5% level) decrease from these percentages during the observation period was scored as −1, any significant increase as +1, and no change as 0. In addition, any observation significantly lower than an immediately preceding observation other than at silking was scored −1; if higher, +1. Thus, possible scores for each observation were +2, +1, 0, −1, and −2. A score of +2 indicated that the observed sugar percentage was significantly higher than the per cent present at silking (+1), and also higher than the per cent present in the preceding observation (+1). The assigned values, totaled, represented the sugar-trend score for each inbred line. This scoring scheme gave more significance to early and rapid changes in sugar concentration than to gradual changes occurring throughout the observation period or to large changes occurring late in the observation period. Table 5 gives the sugar-trend scores and correlation coefficients of the relationship between these scores and stalk rot scores.

The inbred lines used showed a significant negative correlation between increase in sugar levels after silking and susceptibility to stalk rot. High stalk rot scores indicating a high degree of susceptibility were associated with significant decreases in sugar levels. All significant decreases in sugar levels resulted from a decrease in reducing sugars; none of the inbred lines exhibited a significant decrease in sucrose from amounts present at silking. Significant increases in sucrose content were measured in 6 inbred lines. The trend in total sugar (sucrose and reducing sugars) was more closely correlated with stalk rot reaction than trends in either of the component sugars alone (Table 5).

Group sugar-trend scores were obtained by totaling the values for the 4 inbreds in each of the 3 stalk rot reaction classes. The respective scores for sucrose, reducing sugars, and total sugars were: susceptible inbreds—17, 10, and 27; intermediate inbreds—20, 15, and 35; and resistant inbreds—23, 18, and 41.

Pith density.—The inbred lines in the resistant class exhibited a higher density of pith at silking than inbreds in the intermediate and susceptible classes (Fig. 4). The density of pith decreased in resistant lines during the observation period, but was higher at the final observation, except for R130, than any of the less resistant inbreds.

Among the intermediate inbreds, R130 maintained a comparatively high level of pith density throughout the experimental period and was higher in this respect than any other inbred at the final observa-

Table 4.—Percentage of reducing sugars on a dry-weight basis in stalk samples of 12 inbred lines at various intervals after planting.

Sampling date	Rank of inbreds and mean per cent of reducing sugars[a]											
8 weeks after planting[b]	R130 (48.0)	C103 (46.5)	38-11 (40.3)	WF9 (39.0)	R4 (37.0)	R109B (36.7)	R53 (35.3)	B14 (35.3)	C.I. 4-8 (32.0)	R80 (31.7)	R168 (28.0)	R101 (27.3)
At silking	R130 (52.0)	B14 (39.0)	R80 (36.5)	R53 (35.3)	R101 (34.7)	R4 (34.3)	C103 (33.8)	R109B (30.7)	R168 (29.5)	38-11 (26.3)	C.I. 4-8 (22.3)	WF9 (19.3)
2 weeks after silking	R130 (36.5)	R80 (35.0)	B14 (34.2)	38-11 (34.2)	C103 (27.0)	WF9 (26.7)	R53 (26.3)	R168 (24.8)	R101 (22.7)	C.I. 4-8 (22.3)	R109B (22.3)	R4 (21.7)
3 weeks after silking	38-11 (46.7)	R130 (46.0)	B14 (40.3)	WF9 (35.5)	R4 (33.3)	R101 (30.0)	C103 (29.7)	R109B (28.7)	R53 (27.0)	R80 (26.3)	C.I. 4-8 (24.3)	R168 (19.3)
4 weeks after silking	38-11 (49.0)	WF9 (38.5)	B14 (38.3)	C103 (35.3)	R130 (32.5)	C.I. 4-8 (29.0)	R109B (26.5)	R4 (25.7)	R101 (25.3)	R53 (22.5)	R80 (16.5)	R168 (10.0)

[a] See footnote [a] Table 3.
[b] See footnote [b] Table 3.

tion. The remaining intermediate inbreds exhibited lower pith densities at silking than resistant inbreds and maintained the initial densities with minor fluctuations throughout the observation period.

The inbred lines classed as susceptible showed wide variation in pith density at silking, but all were lower than the resistant ones. The susceptible inbreds decreased in pith density during the observation period, and at the final observation had lower pith densities than members of the intermediate and resistant groups.

Significant differences in pith density among inbred lines were determined (Table 6), and correlation coefficients measuring the relationship between pith density and stalk rot scores at different observation dates were calculated (Table 7). With the exception of rot score the first week after inoculation, a significant negative correlation existed be-

Table 5.—Significant changes[a] in percentages of sugars in 12 inbred lines during a 4-week period after silking and correlation coefficients of scores for trends in sugar content and stalk rot rating.

Inbred lines grouped by stalk rot reaction	Sucrose trend scores					Reducing sugars trend scores					Total sugars
	Silking score	Weeks after silking 2	3	4	Sum total	Silking score	Weeks after silking 2	3	4	Sum total	Sum of trend score totals
Susceptible											
R4	+4	0	0	0	+4	+4	−1	+1	0	+4	+ 8
R53	+4	+1	+1	−1	+5	+4	−1	0	−1	+2	+ 7
R80	+4	0	0	0	+4	+4	0	0	−1	+3	+ 7
R168	+4	0	0	0	+4	+4	0	−1	−2	+1	+ 5
Intermediate											
R130	+4	+1	+1	+1	+7	+4	0	0	−1	+3	+10
R109B	+4	0	0	+1	+5	+4	0	0	0	+4	+ 9
C.I. 4-8	+4	0	0	0	+4	+4	0	0	0	+4	+ 8
38-11	+4	0	0	0	+4	+4	0	0	0	+4	+ 8
Resistant											
C103	+4	+1	+1	+1	+7	+4	0	0	0	+4	+11
B14	+4	0	0	+2	+6	+4	0	0	0	+4	+10
R101	+4	0	+2	0	+6	+4	0	0	0	+4	+10
WF9	+4	0	0	0	+4	+4	0	+1	+1	+6	+10
Correlation coefficients of sugar trend scores and final stalk rot scores					−0.650*					−0.595*	− 0.883**

[a] Each inbred was assigned a value of 4 for per cent of the respective sugars present at silking; any significant (5% level) decrease from these percentages during the observations was scored as −1, an increase as +1; in addition any observation lower than an immediately preceding observation, other than at silking, was scored −1; if higher, +1. Thus, possible scores for observations were +2, +1, 0 (no change), −1 and −2.
*Significant at the 5% level.
**Significant at the 1% level.

Table 6.—Pith-core density of the second aboveground internode and rank of 12 inbred lines at silking, and at 2, 3, and 4 weeks after silking.

Weeks after silking	Rank of inbreds and mean pith-core density[a]											
0[b]	B14 (1.05)	WF9 (1.00)	C103 (0.99)	R101 (0.97)	R130 (0.91)	R4 (0.71)	R168 (0.68)	C.I.4-8 (0.65)	38-11 (0.65)	R109B (0.49)	R53 (0.49)	R80 (0.29)
2	R130 (1.05)	C103 (0.98)	WF9 (0.91)	B14 (0.85)	R101 (0.82)	R4 (0.77)	R168 (0.59)	38-11 (0.57)	C.I. 4-8 (0.54)	R109B (0.52)	R53 (0.44)	R80 (0.24)
3	R130 (0.98)	WF9 (0.89)	C103 (0.86)	R101 (0.83)	B14 (0.66)	R4 (0.61)	38-11 (0.58)	R168 (0.55)	C.I. 4-8 (0.50)	R109B (0.49)	R53 (0.35)	R80 (0.28)
4	R130 (1.02)	WF9 (0.83)	B14 (0.80)	R101 (0.78)	C103 (0.71)	C.I. 4-8 (0.67)	38-11 (0.54)	R109B (0.50)	R4 (0.43)	R168 (0.35)	R53 (0.30)	R80 (0.14)

[a] Pith-core density expressed as specific gravity is indicated in (), representing the average of 4 replications; means not underscored by the same line are significantly different at the 1% level.

[b] At silking.

tween pith density on any given date and rot score on or after this date.

Discussion.—A positive correlation was found between resistance to stalk rot and pith density among the inbred lines used. Pappelis (10) has shown that pith density is directly correlated with the per cent of living cells in the pith tissue. These correlations indicate that susceptibility to stalk rot follows the senescence of pith tissue. Consequently, any environmental or plant factor that influences the degree of senescence of pith tissue would also influence invasion by *D. zeae*.

Sugar analysis of the inbred lines showed that a downward trend in sugar level after silking was positively associated with senescence and susceptibility. The decrease in sugar level was accompanied by a decrease in the number of living cells in the pith tissue of the stalk. This is to be expected since carbohydrates are an energy source for the metabolic activities of the cell (2).

Loomis (8) found sugars to be translocated along a negative gradient from the stalk to the developing ear. If the supply of sugar is insufficient to meet the needs of both grain development and maintenance of the vegetative parts of the plant, grain formation is accompanied by a reduction in physiological activity and eventual senescence of these vegetative parts. This senescence, and its associated chemical changes in the pith, result in the plant becoming susceptible to stalk rot.

The observations on increased incidence of stalk rot following leaf damage and of decreased susceptibility following defruiting are in agreement with this theory. Loss in leaf area would decrease the sugar manufacturing potential of the plant, and defruiting would increase the amount of sugar available for vegetative functions.

Comparisons of pith densities among the inbred lines show the susceptible inbreds are lower in density at silking than the resistant ones. These differences in densities at silking cannot be attributed to differences in sugar trends after silking. Since only 1 sugar analysis was made prior to silking, trends for this period were not established. The possibility exists, however, that in some lines sugar levels start to decrease before silking. An alternative possibility would be that differences in density at silking are caused by some factor or factors other than sugar content.

Pith density decreased in the resistant inbred lines during the later part of the observation period, even though these lines showed a general upward trend in sugar level. The increase in sugar possibly failed to meet the requirements both for grain production and for vegetative cell maintenance. Under these circumstances, senescence would occur, but at a slower rate than in inbreds where sugar deficiencies were greater.

Table 7.—Correlation coefficients of pith-core density and stalk rot scores of 12 inbred lines at various weekly intervals after inoculation.

Pith-core density at weekly intervals after inoculation	Stalk rot scores at weekly intervals after inoculation			
	1	2	3	4
—1[a]	—0.530	—0.859**	—0.870**	—0.715**
1	—0.524	—0.873**	—0.911**	—0.771**
2	—	—0.811**	—0.833**	—0.746**
3	—	—	—0.780**	—0.809**

[a] At silking (1 week prior to inoculation).

**Significant at the 1% level.

Further work is needed to determine the reasons for the differential trends in sugars exhibited by the corn inbreds studied. Downward trends could be brought about by a decrease in photosynthetic activity. On the other hand they could indicate that a greater proportion of the sugars produced by the plant was translocated to the ear in inbreds showing decreases in sugar. Koehler (7) reported that, in a group of corn hybrids, those most susceptible to stalk rot were the highest yielding in seasons or locations of low stalk rot incidence. This may indicate that the ultimate in stalk rot resistance may be incompatible with the ultimate in grain yields if stalk rot resistance is achieved only by living cells. The interrelationships of carbohydrate metabolism, yield, and stalk rot reaction certainly deserve more intensive study.—University of Illinois, Urbana.

LITERATURE CITED

1. Association of Official Agricultural Chemists. 1950. Official methods of analysis. Assoc. Offic. Agr. Chemists.
2. Bonner, J. 1950. Plant biochemistry. Academic Press, Inc. Publishers New York, New York.
3. Craig, J. 1960. Physiological, chemical and morphological plant factors in Zea mays L. associated with Diplodia stalk rot reaction. Unpublished Doctoral Thesis. Univ. of Illinois, Urbana, Illinois.
4. DeTurk, E. E., and E. B. Earley. 1937. Resistance of corn hybrids related to carbohydrates. Univ. of Illinois Agr. Expt. Sta. Rept. 40:244.
5. Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11:1-42.
6. Holbert, J. R., P. E. Hoppe, and A. L. Smith. 1935. Some factors affecting infection with and spread of Diplodia zeae in host tissue. Phytopathology 25:1113-1114.
7. Koehler, B. 1960. Cornstalk rots in Illinois. Univ. of Illinois Agr. Expt. Sta. Bull. 658.
8. Loomis, W. E. 1934. The translocation of carbohydrates in maize. Iowa State Coll. J. Sci. 9:509-520.
9. Messaien, C. M. 1957. Richesse en sucre des tiges de mais et verse parasitaire. Rev. pathol. vegetale et entomol. agr. France 36:209-213.
10. Pappelis, A. J. 1957. Nature of resistance to Diplodia stalk rot of corn. Unpublished Doctoral Thesis. Iowa State Univ. Ames, Iowa.
11. Pappelis, A. J., and F. G. Smith. 1960. Nature of resistance to Diplodia stalk rot of corn. (Abstr.) Phytopathology 50:650.
12. Wernham, C. C. 1959. Corn stalk rot trials in Pennsylvania, 1958. Plant Disease Reptr. 43:863-870.

DIPLODIA ROOT AND STALK ROT OF DENT CORN

Jeweus Craig and A. L. Hooker

Respectively Research Associate and Associate Professor of Plant Pathology, University of Illinois, Urbana. Accepted for publication November 14, 1960. Portion of a Ph.D. thesis, University of Illinois.

SUMMARY

Stalk rot in dent corn grown in the greenhouse was induced by infesting the soil with *Diplodia zeae* when the plants were 8 weeks old. The roots were the site of initial infection, and the pathogen progressed into the stalk by way of infected roots. Histological studies were made of infected adventitious roots and of infected stalks. Many invaded cortical cells in the roots exhibited cellular ingrowths or browning and deposition of a granular material. Other cortical cells gave no visible response to hyphal invasion. Vessel elements of infected roots and stalks were occluded with lignin-like substances. Infected nodes were dark brown and exhibited cellular ingrowths. Dead parenchyma cells of the internodes showed no browning or other response to invasion by *D. zeae*. The most damaging aspect of the infection appears to be plugging of the vascular system.

Durrell (2) reported that stalk rot of corn caused by *Diplodia zeae* (Schw.) Lév. resulted from infection of the nodes of the corn plant. From isolation studies, Koehler (6) reached similar conclusions. McNew (9) demonstrated that Diplodia stalk rot could result from infection of the parts of the plant belowground, either from infected seed or from infested soil. He found that the mesocotyl was the point of initial infection. Recently, Whitney and Mortimore (13) reported that stalk rot of field corn in southwestern Ontario began as a root rot that progressed into the stalk. The causal agent, however, was not believed to be *D. zeae*. Other workers (4, 8, 10, 12) have noted an association between root necrosis and stalk rot in corn.

This study was made to determine the pathway of infection by *D. zeae* in corn plants grown in infested soil, and the effect of infection on the tissues of roots and stalks.

Materials and methods.—Twenty-one 10-in. clay pots were filled with soil and steam sterilized. Three 4-in. sections of 18-mm glass tubing, corked at the top end, were inserted in the soil equidistant around the perimeter of each pot. The tubes were angled in so that the corked ends at the edge of the pot were ½ in. above the soil surface and the lower ends were 2½ in. below the surface and near the center of the pot.

Seed of a dent corn single cross Ill. Hy 2 x C.I. 4-8 was treated with bis(dimethylthiocarbamyl) disulfide (Arasan SF-X) and planted at the rate of 3

kernels per pot. The plants were thinned to 1 per pot and placed in a room where light and temperature were adjusted to simulate field conditions in summer. A nutrient solution made by mixing 17 g of 12-24-12 commercial fertilizer with 4 L of water was applied at weekly intervals at the rate of 500 cc per pot. Soil moisture was maintained by watering the plants twice daily.

Inoculum was prepared from 10-day-old colonies of *D. zeae* growing on potato-dextrose agar (PDA). The cultures, 1 Petri-dish culture for each 300 cc of sterile distilled water, were macerated in a Waring Blendor. Eight weeks after planting, 100 cc of the inoculum was poured through the glass tubes into the soil beneath the plants in 14 of the pots. Similar amounts of a sterile mixture of water and PDA were added to 7 control pots.

Infection of roots, crowns, and stalks was observed 8, 12, and 14 weeks after soil infestation. Stalks and roots of the plants selected for observation were carefully removed from the pots and thoroughly washed in water. Stalk and root sections were surface-sterilized with a 5% solution of sodium hypochlorite, and isolations were made by plating bits of this tissue on PDA.

For histological studies of diseased tissue, cross sections about ¼ in. thick were cut from stalks and roots and infiltrated with FAA killing and fixing fluid. Later, thin freehand sections were cut from this material and observed microscopically.

Mycelium in the diseased plant tissue was differentiated by staining with Sudan IV. The sections were washed free of FAA in 50% ethyl alcohol and transferred to a saturated solution of Sudan IV in 70% ethyl alcohol. After 30 minutes in Sudan IV, the sections were quickly washed in 50% ethyl alcohol and mounted in glycerine for observation (5).

Fresh, unfixed material was used in determining, with specific stains, the nature of certain structural characteristics of diseased tissue. Lignified structures in these fresh sections were identified by treatment with phloroglucin (5). Suberin and other fatty substances were differentiated by staining with Sudan IV (5).

Results.—*Root and stalk rot development.*—Eight weeks after soil infestation and 6 weeks after silking, 5 plants from the infested pots and 2 from the check pots were examined. Plants from the check pots showed no lesions on either roots or crowns. Four of the 5 plants from infested pots had dark brown lesions on the adventitious roots belowground. No discoloration was observed on or in the base of the stalk. Bits of tissue from the necrotic areas of the roots were plated on PDA. *D. zeae* and unidentified bacteria were obtained. The plated tissue from the interior of the base of the stalks yielded only unidentified bacteria.

Four weeks later (12 weeks after infestation) 4 plants from infested pots and 2 from check pots were examined. The check plants were free of crown or root rot. Roots of the 4 plants from the infested pots showed large areas of brown rot, and the lesions in 2 of the plants, extended to the junction of stalk and root. The outer surfaces of these stalks were discolored and, when split, revealed a dark discoloration of the basal crown tissues. The first 2 internodes above the soil line also exhibited a dark discoloration of the rind. The internodal vascular bundles were brown, but the internodal parenchyma tissue was not discolored. Nodes in this region were dark brown. Tissues from discolored adventitious roots, nodes, and rinds and from internodal parenchyma tissue showing no discoloration were plated on PDA. *D. zeae* grew from all plated tissues, including internodal parenchyma tissue showing no discoloration.

The remaining plants were examined 14 weeks after *D. zeae* was introduced into the pots. The stalks (Fig. 1) from the infested pots exhibited symptoms the same as those just described. In addition, pycnidia of *D. zeae* were abundant on and in the roots and on the stalk surface above and below the soil line. Pycnidia were also found in the parenchyma tissue of the first 2 internodes above ground level, even though this tissue showed no discoloration.

Histological study.—Microscopic observations were made of sections from the necrotic areas of the roots, crowns, and stalks. In the necrotic region of the roots the cortical cells and intercellular spaces were brown. Hyphae were abundant in many of these cells (Fig. 2-A). Many cortical cells with partially destroyed cell walls were filled with a brown, granular material. Hyphae were not distinguishable in these cells, but there was some indication that mycelium might be present as a compact mass of short segments.

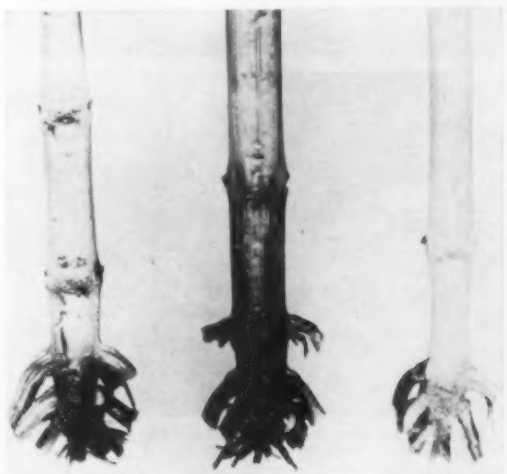


Fig. 1.—Diseased corn stalks (left and center) grown in pots in the greenhouse in soil infested with *D. zeae*, and healthy stalk (right) grown in uninfested soil.

Ingrowths of the cell wall that formed around invading hyphae were observed in many cortical cells (Fig. 2-B). Fellows (3) reported similar ingrowths in cortical cells of wheat roots invaded by *Ophiobolus graminis* Sacc. He named these structures ligni-tubers because of their positive reaction to phloroglucinol, a stain specific for lignin. Young (14) noted such formations in wheat coleoptiles invaded by *D. zeae*, and called them callosities. The ingrowths in the cortical cells of diseased corn roots, however, gave a negative reaction to phloroglucinol. Some of these structures stained pink when treated with Sudan IV. This reaction indicates that these structures could be composed of suberized material. They often extended almost entirely across the cell lumen. In many instances the hyphae succeeded in breaking through the enclosing envelope (Fig. 2-C).

The endodermis appeared to retard the progress of the pathogen. After penetrating this barrier, hyphae of *D. zeae* entered the stele (Fig. 2-D). Occluding material was observed in the vessel elements of the invaded stele (Fig. 2-E). In sections from fresh roots this occluding material stained red when treated with phloroglucinol, indicating that the substance

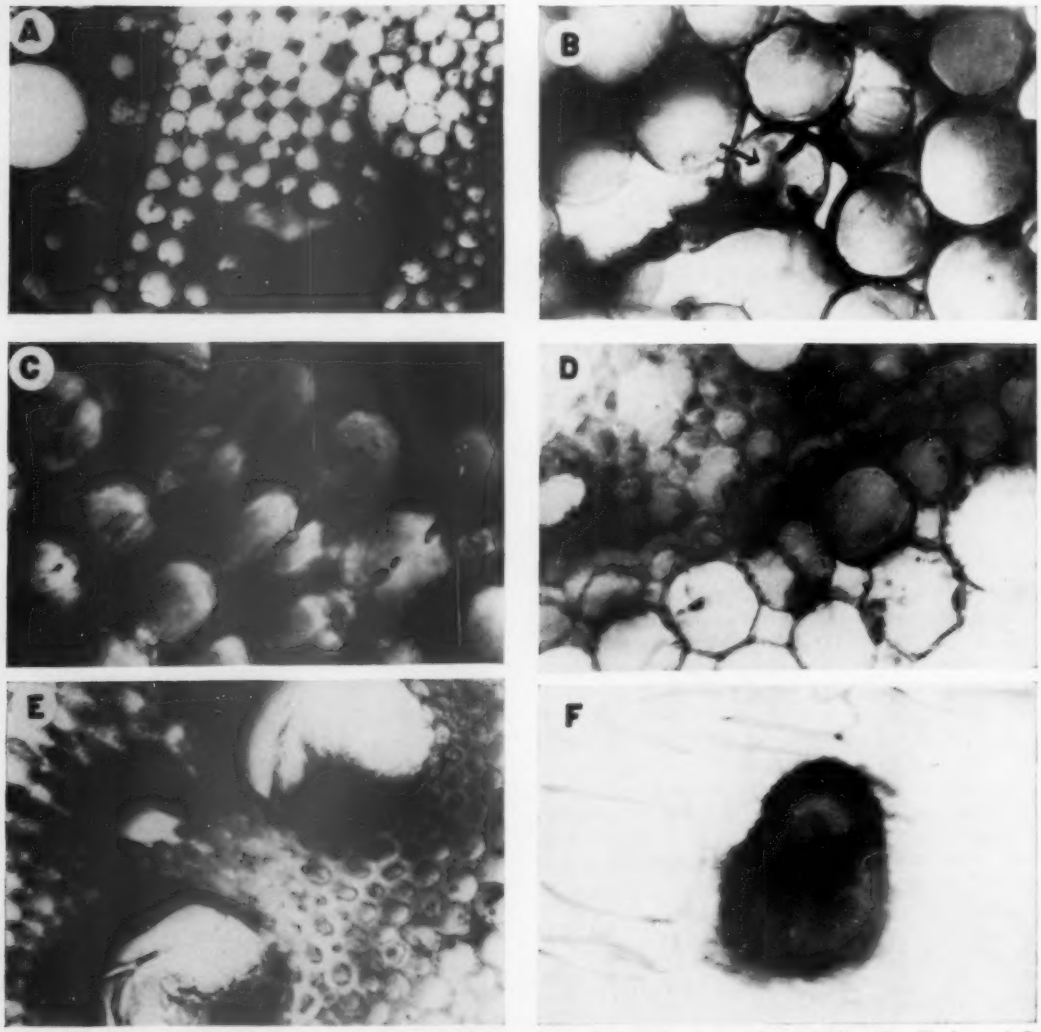


Fig. 2.—Sections from roots and stalks of corn plants infected with *D. zeae*. **A)** Cross section of the necrotic region in the root cortex showing darkly colored cells and intercellular spaces surrounded by cells in which hyphae are discernible (×120). **B)** Cross section of cells in the root cortex, showing ingrowths (arrow) (×400). **C)** Cross section of cells of root cortex, showing hyphae emerging from the cellular ingrowths (×400). **D)** Hyphae of *D. zeae* (discernible as strands and black dots) in cortex and stele of cross section of a corn root (×400). **E)** Partially occluded vessel elements in cross section of the stele of a corn root (×232). **F)** Pycnidium of *D. zeae* in internodal parenchyma cells of a stalk (×232).

was lignin or lignin-like. Often the occluding material found in a vessel element was not accompanied by hyphae.

Fourteen weeks after soil infestation the underground internodes of the stalk, composed almost entirely of vascular tissue, were darkly discolored. A different reaction, however, was evident in the aboveground internodes, where large amounts of parenchymatous tissue were present. In these nodes and internodes, the vascular bundles of the internodes and the cells of the nodes exhibited the browning and occlusion noted earlier in the roots but the parenchyma cells in the inner tissue of the internode showed no browning. Even so, they contained abundant hyphae and pycnidia of *D. zeae* (Fig. 2-F).

Discoloration and occlusions were evident in the cells of the nodal tissues. Cellular ingrowths similar to those found in the roots were abundant. The parenchyma tissue in the internode showed no reaction to invasion by *D. zeae*, but had the dry, spongy appearance reported by Pappelis (11) as indicative of dead cells.

Discussion.—Under the conditions of this experiment, *D. zeae* caused stalk rot by invading the roots of the corn plant and progressing into the stalk. It is recognized that the method of inoculation employed in the present study was extremely severe and highly unnatural. *Diplodia* may not readily infect corn roots growing in unsterile field soil, where levels of *Diplodia* are lower and competition with numerous soil organisms exists. Nevertheless, this experiment demonstrates that *Diplodia* stalk rot can be initiated by root infections from the soil. Whether stalk rot as found in the field follows this pattern or, as others (2, 6) have reported, results from aboveground infections cannot be definitely ascertained without further experiment.

Observations of the diseased stalks in this study showed that nodal tissues aboveground were distinctly discolored both on the surface and in the interior of the stalk. The internodal parenchymatous tissue above and below these nodes showed little or no discoloration. Thus, an investigator observing only these symptoms might conclude that primary infection occurred at the discolored nodes rather than progressing upward from the base of the plants.

The internodal parenchymatous tissue whose cells gave no visible response to invasion by *D. zeae* appeared to be composed of dead cells. If cell browning or formation of cellular ingrowths in response to hyphal invasion are the properties of living cells, the parenchyma cells in the nodes retained their viability longer than those in the internodes.

The stage at which roots become susceptible was not determined, but the great increase in the portion of the root system infected at 8 weeks over that at 4 weeks after silking indicates that susceptibility to root rot by *D. zeae* increases with plant maturity. Craig and Hooker (1) and Pappelis (11) have reported that susceptibility to *Diplodia* stalk rot resulting from artificial inoculation is associated with the degree of senescence of stalk tissue. Loomis (7) found that the root growth of the corn plant was sharply curtailed after silking. In view of these findings, the susceptibility of the roots to infection may result from progressive senescence of the cortical tissue following silking.

Histological observations revealed that invasion of the vascular system by *D. zeae* caused a plugging of the vessel elements. The occluding material in the vessel elements gave a lignin-like reaction to phloroglucinol, suggesting that the occlusion resulted from deterioration of the walls of the vessel elements. In many instances no hyphae were apparent in the occluding material. Perhaps this material was translocated from some previously invaded point. There is also the possibility that an exotoxin produced by the pathogen could initiate the formation of occluding material in advance of the mycelium.

Plugging of vessel elements in the roots would impair the translocation of water and nutrients from the roots to the upper portions of the plant. If this impairment of translocation was severe enough, premature dying of stalk, which is often associated with infection by *D. zeae*, would result. Even when root damage is not severe enough to cause premature dying, infection would result in a decrease in plant vigor and consequent reduction in grain yield.—University of Illinois, Urbana.

LITERATURE CITED

1. Craig, J., and A. L. Hooker. 1961. Relation of sugar trends and pith density to Diplodia stalk rot in dent corn. Phytopathology 51:376-382.
2. Durrell, L. W. 1923. Dry rot of corn. Iowa State Coll. Agr. Expt. Sta. Research Bull. 77.
3. Fellows H. 1928. Some chemical and morphological phenomena attending infection of the wheat plant by Ophiobolis graminis. J. Agr. Research 37:647-661.
4. Hooker, A. L. 1956. Association of resistance to several seedling, root, stalk, and ear diseases in corn. Phytopathology 46:379-384.
5. Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill Book Co. Inc. New York, New York.
6. Koehler, B. 1960. Cornstalk rots in Illinois. Univ. of Illinois Agr. Expt. Sta. Bull. 658.
7. Loomis, W. E. 1934. The translocation of carbohydrates in maize. Iowa State Coll. J. Sci. 9:509-520.
8. McKeen, W. E. 1953. Preliminary studies of root and basal stalk rot of maturing corn in Ontario. Can. J. Botany 31:132-141.
9. McNew, G. L. 1937. Crown infection of corn by Diplodia zeae. Iowa State Coll. Agr. Expt. Sta. Research Bull. 216.
10. Melhus, I. E. 1934. Diplodia dry rot of corn. Iowa State Coll. Agr. Expt. Sta. Rept. on Agr. Research p. 75.
11. Pappelis, A. J. 1957. Nature of resistance to Diplodia stalk rot of corn. Unpublished doctoral thesis, Iowa State Univ. Ames, Iowa.
12. Semeniuk, G. 1948. Study of Diplodia dry rot of corn. Iowa State Coll. Agr. Expt. Sta. Rept. on Agr. Research p. 370.
13. Whitney, N. J., and C. G. Mortimore. 1957. Root and stalk rot of field corn in southwestern Ontario. Can. J. Plant Sci. 37:342-346.
14. Young, P. A. 1926. Penetration phenomena and facultative parasitism in Alternaria, Diplodia and other fungi. Botan. Gaz. 81:258-279.

THE DEVELOPMENT OF BLACK SHANK IN TOBACCO AS INFLUENCED BY HOST NUTRITION

J. L. Apple

Accepted for publication November 18, 1960. Published with the approval of the Director of Research, North Carolina Agricultural Experiment Station, as Paper No. 1234 of the Journal Series.

The author expresses appreciation for the technical assistance of C. W. Bennett, Mrs. Alice Hisada, J. W. Hendrix, and J. M. Sledge.

SUMMARY

The effect of varying levels of N, P, and K on predisposing plants of a black-shank-resistant tobacco variety (Coker 139) to *Phytophthora parasitica* var. *nicotianae* was studied. Greenhouse plants grown in sand cultures with high levels of N were more susceptible than those with optimum-growth levels of N, P, and K. The disease indices were consistently less for low-nitrogen plants than optimum-nitrogen plants. High P had little effect on susceptibility but low-P plants were less susceptible than plants receiving optimum-growth levels of N, P, and K. Different levels of K had little effect on susceptibility.

Increased disease incidence or high susceptibility was correlated with high osmotic levels. Plants with above-optimum levels of N, P, and K were more susceptible than optimal-level plants. The results appeared unaffected by day length since data were similar in both summer and fall.

The black shank disease of tobacco, caused by the soil-borne fungus *Phytophthora parasitica* (Dast.) var. *nicotianae* (B. de Haan) Tucker, is controlled mainly by using disease-resistant varieties. In most cases, varieties having moderate to high resistance provide adequate control under field conditions; in some instances, however, losses are heavy. It is known that plant-parasitic nematodes may predispose resistant plants to disease (8) and that highly virulent strains of the fungus may cause considerable damage to resistant varieties (3). In addition heavy losses occur in resistant varieties that cannot be explained on either basis.

There are many reports of predisposing plants to disease by varying mineral nutrition in sand culture (1, 4, 5, 9). There is the possibility, therefore, that the major-element nutrition (N, P, and K) or micronutrient supply may influence black shank development in even a resistant variety. This study was made to determine the influence of major-element nutrition on the predisposition to *P. parasitica* var. *nicotianae* of resistant tobacco plants grown in sand culture.

Methods and Materials.—Plants of the black-shank-resistant variety Coker 139 were grown in sterilized sand in thumb-pots and supplied daily with a balanced nutrient solution favorable for the growth of tobacco. When in the 4-5-leaf stage, 2 plants were transplanted to each varnished 10-in. clay pot containing washed quartz sand (grade 3 Q-ROK, Pennsylvania Glass Sand Corporation). A subirrigation sand culture apparatus was used, consisting of 4 tables, each supporting 12 10-in. pots. The nutrient solutions were stored in 20-L plastic carboys under the tables. Each container was fitted with a rubber tube connected to an air pump and a plastic tube connected to 2 pots on the table above by a 1-hole rubber stopper inserted into the bottom of the pots. The open end of the tube protruding into the pot was covered with a fine mesh plastic screen to prevent clogging. The pots were flooded 1-3 times daily, depending on plant size, by creating air pressure in the carboys. The excess nutrient solution was allowed to drain back into the containers. Six solution treatments were studied in each run, and each was replicated 8 times, with a single 10 in. pot containing 2 plants constituting a replicate. The nutrient solutions were replenished weekly.

Twelve nutrient-solution treatments were studied, involving variables of N, P, and K in various combinations. These treatments and the ppm of the variable elements are shown in Table 1. Mineral-element levels are designated as 0X, the low level; 1X, the optimum level; 2X, twice the optimum level; etc. Thus, a solution treatment designated 111 indicates optimum levels of N, P, and K. The ppm of N and K used as the optimum (1X) agrees with the findings of McEvoy (7), but the level of P (95 ppm) is somewhat lower. Preliminary tests, with green weights measured, indicated that the lower rate of P gave optimum growth. Nitrogen was supplied as nitrate except in treatments 211 and 110, which, respectively, contained 14 ppm and 7 ppm of the ammonium form. Micronutrients were added to all solutions in the following amounts: Mo, 9.5 ppm; Cu, .02 ppm; Zn, .05 ppm; Mn, .5 ppm; Bo, .5 ppm; and Fe, 1.1 ppm.

After the plants were cultured on treatment solution for 4 weeks, each was inoculated with 100 ml of inoculum placed 2 in. from the base of the plant in a hole 1 in. in diameter and 4 in. deep. The inoculum was grown on sterile moistened whole oats and prepared by blending 100 g of infested oats with 1100 ml of water in a Waring Blendor. Greenhouse temperatures after inoculation were 88±10°F.

Disease development data were recorded 15 days after inoculation on a scale of 0-5. Table 2 shows the class descriptions and the class values used in computing the disease indices. Each experiment, consisting of 6 treatments (solutions), was repeated once except for experiment 4. The data from the 2 runs of a given experiment were combined for analysis as a split-plot, with runs as the main effect and treatments as the second-order effect. The experiments were conducted throughout the year; thus, day-length and average daily temperature were greater in summer.

Results.—Treatments that encouraged succulence

Table 1.—Composition of treatment solutions.

Treatment[a]	Elements (ppm)							
	N	PO_4	K	Ca	Mg	SO_4	Na	Cl
111	168	95	235	163	49	192	0	36
011	28	95	235	160	49	384	0	248
211	336	95	235	163	146	192	69	4
101	168	0	235	163	49	192	0	71
121	168	190	235	163	49	192	0	4
110	168	95	20	163	91	192	0	4
112	168	95	469	163	49	384	0	106
222	336	190	469	163	146	192	0	71
100	168	0	20	163	91	192	0	4
102	168	0	469	163	49	384	0	142
333	504	285	704	163	176	192	127	36
114	168	95	938	163	49	864	0	213

[a] The major elements given in the order NPK 0 = low; 1 = normal rate; 2 = double normal rate; etc.

tended to predispose Coker 139 plants to infection by the black shank fungus. The optimum rate (treatment 111) had a consistently high disease index (Fig. 1), exceeded significantly in only 1 instance—by the 222 solution (Fig. 1-B). Treatment 333 in experiment 4 (Fig. 1-D) had a higher index than 111, but the difference was not significant. Thus, when N was used at high levels, either in combination with the optimum or higher levels of P and K, highly susceptible plants resulted.

The low-N treatment (Fig. 1-A, C) reduced susceptibility significantly as compared to other N treatments. Growth was poor and the plants were hard and chlorotic. The average green top weight per plant was 75 g, compared to 233 g for treatment 111.

High P (treatment 121) had little effect on susceptibility as compared to the optimum level (Fig. 1-A, C) but was inconsistent in experiment 4, in which it had the lowest disease index (Fig. 1-D). Low P (treatment 101) reduced susceptibility as compared to the optimum rate (Fig. 1-A,C). These plants were small and dark-green to slightly purple, typical of P-deficient plants.

Four levels of K were included in Experiment 4: 0X, 1X, 2X, and 4X (Fig. 1-D). The 2X level had a significantly lower disease index than 1X with no differences among other levels. All but the 1X level were significantly lower than the 333 solution in the same experiment. Plants grown at the 2X level of K in experiment 2 had a lower index than 1X, but the difference was not significant.

Three K levels (0X, 1X, and 2X) were compared in combination with low P in experiment 3 (Fig. 1-C), with no significant differences. All, however, had significantly lower indices than the 1X balanced solution in the same experiment.

There was no interrelationship between disease development, host nutrition, and other environmental factors such as day length and temperature, insofar as the latter was maintained above 75°F. Experiment 1 was run during August-September and repeated during October-November. In a combined analysis of variance of the 2 runs, the interaction of treatment × runs was not significant. Likewise, experiment 3 was run during June-July and repeated during October-November, with no interaction of treatment × runs.

An agar medium was prepared from each solution treatment by adding 18 g agar to 1 L of nutrient solution. These media were used in culturing *P. parasitica* var. *nicotianae*. Growth was poor on all media, as is characteristic of this organism on a synthetic medium, but there were no significant differences between media.

Discussion.—Nitrogen nutrition was the most important of the 3 macro-elements studied in predisposing black-shank-resistant Coker 139 plants to infection by the black shank fungus. Plants deficient in nitrogen were significantly less susceptible than plants grown at the optimum or higher levels of N fertilization. Plants receiving excessive nitrogen had a higher disease index than plants grown at the normal rate, but the difference was usually not significant. Under field conditions, high rates of nitrogen fertilizer are used with tobacco. In North Carolina the recommended rate of nitrogen fertilization for flue-cured tobacco is 20-70 lb per acre, the specific amount depending on soil type and the crop rotation sequence (2). Many farmers, however, exceed this recommendation by a considerable amount in an effort to get maximum yields under the acreage control program. Consequently, the response to different levels of N in these greenhouse sand-culture experiments could have field significance in terms of the tobacco disease problem in resistant varieties.

Table 2.—Disease classes and class values used for computing disease index.

Class	Class value	Class description
0	0	Healthy
1	6.25	Few fibrous roots diseased
2	12.5	Numerous fibrous roots diseased
3	25.0	Slight infection of primary roots
4	50.0	Tap root diseased; about ½ of root system involved (plants with cortical infection of main stem also placed in this class).
5	100.0	Plant dead (plants placed in this class if pith tissue necrotic).

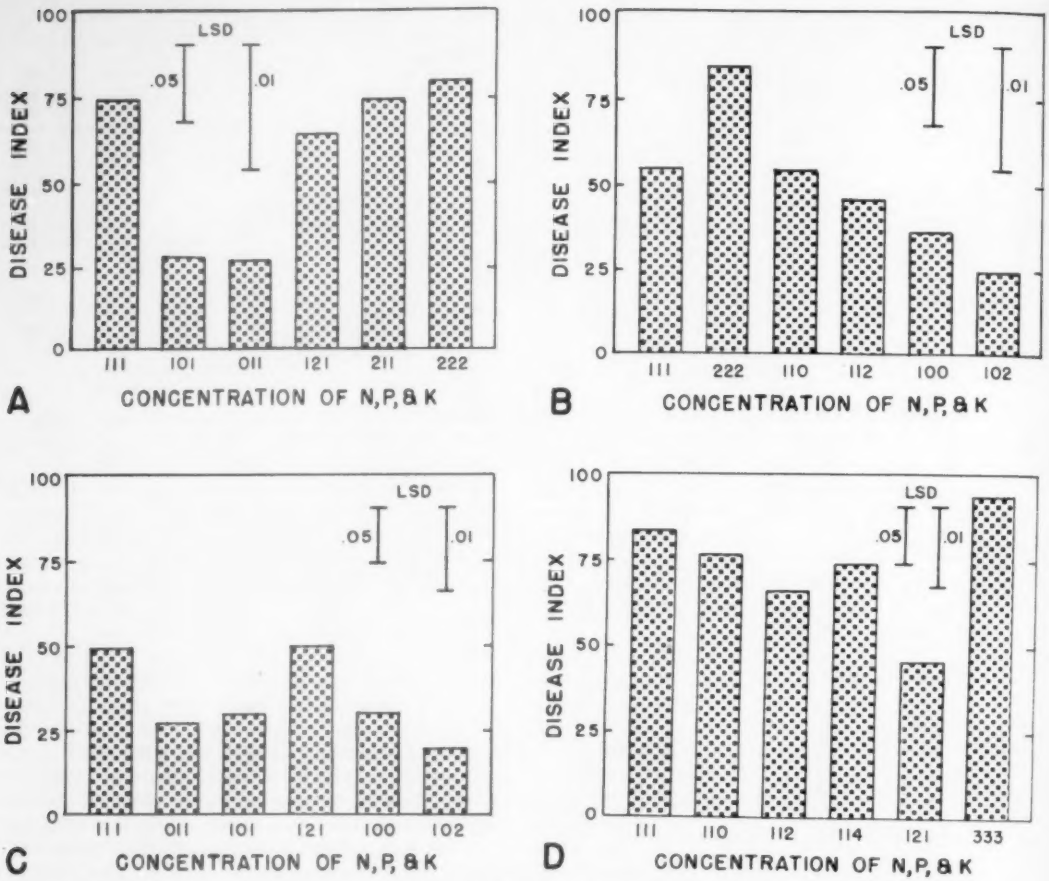


Fig. 1.—Disease indices of plants of the black shank resistant Coker 139 tobacco variety inoculated with *P. parasitica* var. *nicotianae* as influenced by varying levels of N, P, and K in sand culture. The plants were grown on the solution treatments for 4 weeks before inoculation and the data were recorded 15 days after inoculation on a rating scale of 0-5: 0, healthy; 1, few fibrous roots diseased; 2, numerous fibrous roots diseased; 3, slight infection of primary roots; 4, tap root diseased, approx. ½ of root system involved; and 5, plant dead. Class values used in computing the disease indices were, respectively: 0, 6.25, 12.5, 25.0, 50.0, and 100.0. **A)** Experiment 1, combined analysis of 2 runs; **B)** experiment 2, combined analysis of 2 runs; **C)** experiment 3, combined analysis of 2 runs; **D)** experiment 4, data from a single run.

Phosphorus nutrition appears unimportant in predisposing plants to infection. High rates of P did not increase susceptibility, and low P reduced susceptibility. North Carolina soils are inherently low in available P, but 75% of those devoted to tobacco culture for a number of years have medium to high levels of available P (10). Thus, P nutrition likely does not contribute to this field disease problem.

Potassium nutrition had little effect on disease susceptibility. Since many North Carolina soils are inherently low in available P_2O_5, K was varied in a solution series having low P. The low P alone reduced susceptibility, but the K variable was not significant.

The difference in the natural day length did not affect predisposition, as has been reported for other diseases (6). Experiment 3, run with average day lengths of both 14.5 and 11 hours, showed no interaction between treatments and runs.

Anderson (1) and Bingham et al (4) found that the incidence of pineapple and avocado root rots (caused by *P. cinnamomi*) was inversely related to the salt concentration of the nutrient solution. They ascribe this effect to inactivation of the zoospores of the fungus. In the present study, high disease incidence was correlated with high salt concentration; however, the highest levels used were lower than those reported by Anderson and Bingham et al.

LITERATURE CITED

1. Anderson, E. J. 1951. Effect of nutrient salt concentration on infection of pineapple roots by Phytophthora cinnamomi. (Abstr.) Phytopathology 41:2.
2. Anonymous. 1959. Recommendations with reference to the fertilization of flue-cured tobacco in Virginia, North Carolina, South Carolina, Georgia, and Florida. Agronomy Section, Sixteenth Tobacco Workers Conference, Raleigh, N. C. Mimeographed.
3. Apple, J. L. 1957. Pathogenic, cultural, and physiological variation within Phytophthora parasitica var. nicotianae. Phytopathology 47:733-740.
4. Bingham, F. T., G. A. Zentmyer, and J. P. Martin. 1958. Host nutrition in relation to Phytophthora root rot of avocado seedlings. Phytopathology 48:144-148.
5. Castano, J. J., and M. F. Kernap. 1956. The influence of certain plant nutrients on infection of soybeans by Rhizoctonia solani. Phytopathology 46:326-328.
6. Gallegly, M. E., and J. C. Walker. 1949. Relation of environmental factors to bacterial wilt of tomato. Phytopathology 39:936-946.
7. McEvoy, E. T. 1945. Studies on the optimum nutrition of tobacco. Sci. Agr. 25:489-498.
8. Sasser, J. N., H. R. Powers, Jr., and G. B. Lucas. 1953. The effect of root-knot nematodes (Meloidogyne spp.) on the expression of black shank resistance in tobacco. (Abstr.) Phytopathology 43:483.
9. Walker, J. C., and J. B. Kendrick, Jr. 1948. Plant nutrition in relation to disease development. IV. Bacterial canker of tomato. Am. J. Botany 35:186-192.
10. Welch, C. D., and G. D. McCart. 1955. Fertility status of North Carolina soils. II. Publication of the N. C. Department of Agriculture.

EFFECT OF HOST EXUDATES ON CHLAMYDOSPORE GERMINATION OF THE BEAN ROOT ROT FUNGUS, FUSARIUM SOLANI F. PHASEOLI

Milton N. Schroth and William C. Snyder

Respectively Research Assistant and Professor of Plant Pathology, Department of Plant Pathology, University of California, Berkeley.

This research was supported in part by funds from the United Fruit Company and from the W-38 Federal Western Research Project.

Accepted for publication November 21, 1960.

SUMMARY

Chlamydospores of *Fusarium solani* f. *phaseoli* germinated most consistently in the soil when in close proximity to germinating bean seeds and root tips of primary, lateral, and adventitious bean roots. Mature roots had little effect on chlamydospore germination and growth when tested in soil. All were determined by growing beans on filter paper. Exudation of amino acids and sugars was most abundant from germinating bean seeds and root tips. Only traces of exudate were detected from mature roots unless they were dried or injured. An analysis of bean exudates was made to determine which fractions stimulated germination and growth of chlamydospores. Solutions of aspartic acid, glutamic acid, asparagine, glucose, and sucrose—and to some extent, maltose and fructose—stimulated germination and growth when tested in vitro. All were identified as constituents of bean exudate. Analysis of hypocotyl exudate indicated the presence of sugars but only trace amounts of amino acids. It is postulated that amino acids and sugars of bean exudate favorably influence germination and growth of chlamydospores in soil.

INTRODUCTION.—Field evidence indicates that *Fusarium solani* (Mart.) App. & Wr. f. *phaseoli* (Burk.) Snyd. & Hans. is limited in its saprophytic growth in soil and exists in the form of thick-walled chlamydospores imbedded in plant debris or particles of organic matter (10). The organism is a cortical root- and stem-rotting soil-borne fungus pathogenic to bean (*Phaseolus vulgaris* L.), and its principal growth in soil is associated with its pathogenic activities. The fungus normally attacks the bean plant by multiple infections of roots and hypocotyl (20). This implies that growth of the fungus has taken place in the soil in proximity to the infection court from dormant chlamydospores.

This paper reports on the role of plant exudates on the germination and growth of chlamydospores of *F. solani f. phaseoli.*

MATERIALS AND METHODS.—*Germination of chlamydospores near plant parts.*—The soil used was a sandy loam from a bean field in the Salinas valley. The amount of inoculum in the soil was increased by adding conidia of pathogenic clones of *F. solani* f. *phaseoli* and incubating it (several months) until the fungus existed as dormant chlamydospores. Pinto bean seeds were planted in plastic containers with removable sides to facilitate examination of soil adjacent to growing plant parts. The soil was examined at intervals, 16 hours after planting, by placing a small aliquot on a slide, moistening with water, and spreading evenly over the surface. After drying and the removal of the larger mineral fragments, the slide was stained with acid fuchsin, covered with a cover slip, and examined for germinated chlamydospores.

Detection of regions of exudation.—A 7 x 10-in. sheet

of Whatman No. 3MM filter paper was inserted between two 7 x 10-in. sheets of aluminum foil and autoclaved 15 minutes at 250°C. Pinto bean seeds were treated in a solution of 10% Clorox (sodium hypochlorite) and 2% ethanol for 3 minutes. The alcohol aided in the detection of cracked seed coats. Bean seeds with intact seed coats were placed 1½ in. apart near an edge of the paper. The paper was moistened with sterile distilled water and placed in a sterilized 1 x 10 x 8-in. plastic container. Sterilized nonabsorbent cotton was used to press the foil snugly against the beans to encourage the growing roots to grow flush with the paper. The container was inserted into a polyethylene bag, placed in the dark, and canted at a 60° angle to induce the roots to grow both downward and against the paper. Materials exuded from plant parts were absorbed by the filter paper. After 5 days, the germinated beans were removed and tested for contamination by placing them in petri dishes containing potato-dextrose agar (PDA). The filter paper was dipped in ninhydrin color reagent (14) to detect the exudation of amino acids, amides, and amines. Silver nitrate (12) was used to detect the exudation of reducing sugars. Fifty replications were made, with 30% discarded because of contamination.

In further studies, bean seedlings grown as described were transferred to sterile containers containing fresh sheets of filter paper. The bean seedlings were subsequently removed after 6, 12, and 36 hours, and the papers developed with the color reagents. The regions of exudation were determined by comparing the growth and location of plant parts with the patterns of exudation exhibited on the paper. To test the possibility that hypocotyls exuded substances, excised hypocotyls were paraffined at the ends to prevent bleeding, and processed in the manner described above.

Bioassay of amino acid and sugar constituents of bean exudate.—Bean exudates were collected and fractionated to determine which component stimulated germination and growth of chlamydospores of *F. solani* f. *phaseoli*. Clorox-treated bean seeds with intact seed coats were placed in sterile 500-ml flasks, 3 per flask, containing moistened purified sand that had been acid washed and ignited. After 10 days, each flask was tested for contamination by placing segments of bean seedlings and a small amount of sand in petri dishes containing PDA. The contents of 10 flasks, which had remained sterile, were washed several times with distilled water. The washings were filtered to eliminate root detritus and evaporated *in vacuo* to 10 ml. The concentrate was adjusted to pH 5.0 with 1*N* HCl and passed through a column of Dowex 50W-X8, 200-300 mesh, H^+ form, a cationic exchange resin, according to the method of Plaisted (13). The eluate was evaporated to dryness and dissolved in 2 ml of 10% isopropanol in water. Amino acid determinations were conducted by spotting 40 μl of the concentrate on Whatman No. 1 filter paper and running 2-dimensional chromatograms by methods similar to those of Porter et al (14). Sugar determinations were made by the methods of Partridge (12). The fraction that passed through the exchange column was evaporated *in vacuo* to 2 ml, and 30 μl of the concentrate spotted on Whatman No. 1 filter paper.

Identification of amino acids and sugars was made by Rf values, patterns on the chromatograms, colors of the amino acids, different solvent systems, and known and unknown chemicals run simultaneously.

Hypocotyl exudates were similarly fractionated and the constituents identified. The exudates were collected by excising the aboveground portion of 50 hypocotyls of 15-day-old Pinto bean plants. After paraffining the ends and immersing in a solution of 7% Clorox for 3 minutes, they were placed in a sterile moist chamber. After 30 hours, the hypocotyls were rinsed in distilled water and the washings analyzed by the procedures previously described.

Each identified chemical was assayed for activity in stimulating germination and growth of chlamydospores of *F. solani* f. *phaseoli* by pipetting 0.2 cc of 2% aqueous solution into 0.5 g of air-dried soil in a well of a porcelain spot plate and incubating 24 hours in a moist chamber. The solutions were prepared from standardized reagents. Samples were examined after 16 hours. The soil and examining techniques were identical to those used for testing the effect of plant parts on germination of chlamydospores. Chemicals

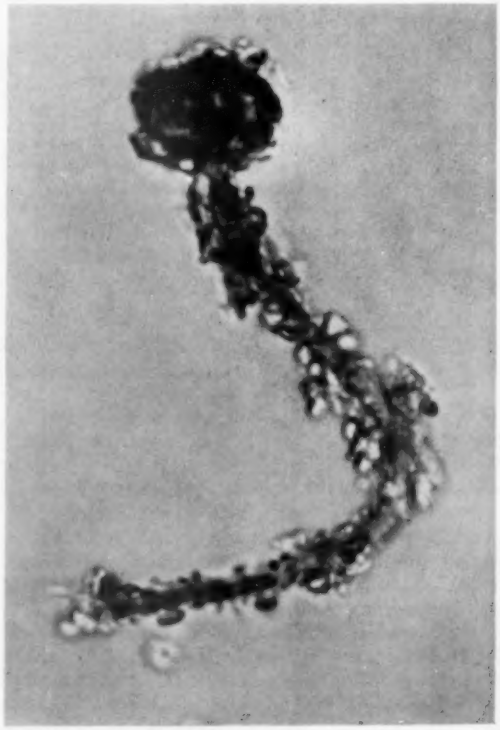


Fig. 1. Germinated chlamydospore of *Fusarium solani* f. *phaseoli* stimulated by a bean root tip (Pinto). Particles clinging to the germ tube are mineral fragments and organic matter.

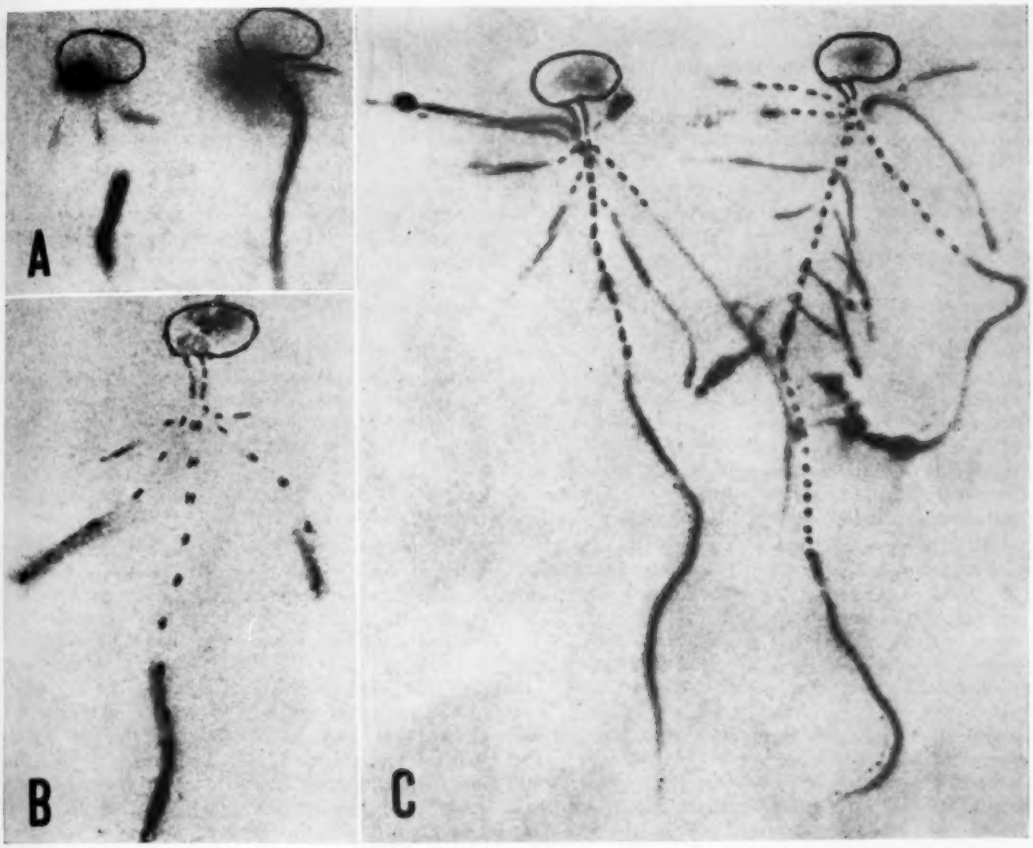


Fig. 2. Exudation patterns of bean plants (Pinto) obtained by growing a bean seedling on Whatman No. 1 filter paper and developing the paper with ninhydrin. **(A)** Exudation patterns of 5-day-old bean seedlings. Original position of ungerminated seeds outlined. **(B-C)** Regions of exudation after transferring 5-day-old bean seedlings to fresh sheets of filter paper and growing for 12 and 36 hours, respectively. Root systems and positions of germinated seeds at time of transference outlined. Note that exudation was primarily from the growing root tips.

showing activity were reassayed in lower concentrations and combinations to test the effect of concentration on chlamydospore germination and growth. Thirty replications were made of each concentration.

RESULTS.—*Effects of plant parts on chlamydospore germination.*—Germination of chlamydospores was observed 16-24 hours after planting bean seeds in moist soil (Fig. 1). The stimulus affecting chlamydospore germination appeared to be confined to a peripheral zone about 1 mm deep around the bean seeds and growing roots, especially by the root tips of primary, lateral, and adventitious roots. Germination was not detected outside this zone. Mature roots appeared to have little effect on chlamydospores unless the roots were injured.

Regions of exudation.—Development of the filter paper with ninhydrin showed that exudation was primarily from germinating seeds and root tips of primary, laterai, and adventitious roots. The exudation of ninhydrin-reacting materials appeared to be particularly heavy from bean seeds (Fig. 2-A). Most of the exudation occurred during the early stages of germination. Root exudation was principally from the growing root tips as evidenced by the trail of exudate (Fig. 2-B,C). The source of the exudate appeared to be in the vicinity of the apical meristem, region of elongation, and region of developing root hairs. Only traces of exudate were detected from mature roots unless they were injured or dried. The development of lateral roots provided a fresh source of exudate in the region of mature roots. Adventitious roots erupting from the hypocotyl also yielded exudates.

Similar patterns of exudation were obtained by developing the filter paper with color reagents specific for sugars. Only trace amounts of hypocotyl exudate were detected when excised hypocotyls were laid on filter paper. Nevertheless, an analysis of the wash water from 40 excised hypocotyls indicated that there

was an exudation of glucose, fructose, sucrose, and maltose.

Effect of amino acids and sugars on chlamydospores.—Germination and growth of chlamydospores of *F. solani* f. *phaseoli* were stimulated by adding to the soil solutions of aspartic acid, glutamic acid, asparagine, glucose, and sucrose—and to some extent, fructose and maltose. All had been identified as constituents of bean exudate and were present in easily identifiable quantities. The application of each chemical to the soil at a concentration of 1%, excluding maltose and fructose, resulted in the germination of about 20-40% of the chlamydospores. Maltose and fructose stimulated the germination of about 10-20% of the chlamydospores. No germination was observed in the controls. Percentage of germination of chlamydospores varies with such variables as soil type, degree of fungistasis, clonal characteristics, and age and viability of the chlamydospores. Concentrations lower than 1% resulted in markedly lower percentages of germination. By combining the amino acids and sugars the concentration could be lowered to 0.2% without any noticeable decrease in germination.

Discussion.—Apparently pathogenesis and survival of *F. solani* f. *phaseoli* in the soil are largely dependent on association with underground plant parts. The growing bean plant helps to induce infection by its growth into the micro-environment of dormant chlamydospores. As the plant grows through the soil, it alters the micro-environment as evidenced by the germination and growth of the dormant chlamydospores. The behavior of these chlamydospores approaches Garrett's (4) description of an ideal resting spore: one that will not germinate under environmental fluctuations in the soil in the absence of the host root, but that is sufficiently sensitive to host root excretions to ensure rapid germination when stimulated. The lack of such a stimulus or nutriment seems to favor dormancy, which may be a factor enhancing survival of *F. solani* f. *phaseoli* until a suitable substrate, such as a bean plant, is present. Pathogenesis is consequently dependent on the mass and distribution of inoculum in the soil. Infection will take place only if the inoculum is close to the underground parts of the bean plant.

Brown (1) advanced the hypothesis that to germinate, dormant spores may need an external supply of an activator because of a failure to synthesize the substance or analog necessary for germination. Chlamydospores of *F. solani* f. *phaseoli* germinate in distilled water, sterile soils, and water agar, but not in field soil: this suggests that germination may involve an overcoming or masking of fungitoxic agents in the soil. Nutrient materials have been observed to reduce the apparent toxicity in the soil (7, 16, 18). It appears that the growing bean plant provides the nutriment materials and environmental influence necessary to overcome fungistasis. The amino acids and sugars that stimulated germination and growth of chlamydospores *in vitro* were constituents of this material and were present in substantial amounts. Dobbs and Hinson (3) similarly found that unsterilized soil inhibited the germination of spores of *Penicillium frequentans* Westling and that germination could be stimulated by using concentrations of glucose as low as 0.1%. Spore germination with *Urocystis tritici* Körn, the cause of flag smut of wheat, is stimulated by a variety of substances and by the roots of several nonhosts as well as host plants (11).

Although bean root tips appear to be a principal source of exudate, the fungus lesions appear on mature roots. Presumably, as the root tip grows past a chlamydospore the chlamydospore germinates, forms a thallus, and infects the maturing root. It appears that continued saprophytic growth of the fungus is contingent upon the quantity of nutrient present, and the capability of the fungus to resist fungistasis. The growing mycelium may be less sensitive to fungistasis since some fungitoxic materials have been reported to be generally more active against spore germination than against growth or metabolism of mycelium (5, 15, 19, 21, 22). Root tips have also been observed to have a stimulating effect on phanerogamic parasites and nematodes (2, 8, 23, 24).

The exact source of root exudate is problematical. Some of the exudate probably consists of materials liberated by sloughing and autolysis of cells, and physical damage to roots. Root hairs may be a source of exudate since reports show root hairs are capable of maintaining absorption and exudation at the same time (9). This exudation supposedly occurs mostly in the region of growing root hairs. Roberts (17) claimed that under certain conditions, the tips of young root hairs would burst, eject their contents, and close immediately, leaving little evidence of the rupture. An analysis of hypocotyl exudate revealed only trace amounts of ninhydrin-reacting substances, but clearly indicated the presence of the same sugars that stimulated germination and growth of chlamydospores when tested *in vitro*. From field observations, it appears that hypocotyls are more severely infected at the basal region, especially early in the season. This is in line with the findings reported here that exudation in this region is particularly heavy. Initially, there is a heavy exudation from the germinating bean seed. Thereafter, the region receives exudate from the growing root tips of primary, lateral, and adventitious roots.

Since amino acids and sugars have been identified in the exudates of many plants, the question arises as to the effect of nonhost exudates on the survival and saprophytic growth of *F. solani* f. *phaseoli*. The pathogen appears to survive readily for long periods in the absence of host plants, and the possibility arises that survival may be influenced by the ability of the fungus to multiply in the rhizosphere of nonhost plants. Plate counts have supported this possibility, for F. F. Hendrix (personal communication) has shown that *F. solani* f. *phaseoli* is more abundant in the rhizospheres of several nonhost plants than in soil free from the influence of the roots. Germinating seeds of several nonhost plants have been observed to stimulate germination and growth of chlamydospores of *F. solani* f. *phaseoli*. Jackson (6) reported that pea, wheat, and

lettuce roots stimulated spore germination of *F. solani* and other *Fusarium* spp.

The role of plant exudates in parasitism becomes increasingly significant with investigation. It is now evident that the growth of *F. solani* f. *phaseoli* is limited to the rhizosphere of bean plants and possibly various nonhost plants, or other temporary supplies of nutrients in the soil. Pathogenesis results from the chance association of dormant chlamydospores with the underground parts of the growing bean plant. The diffusion of materials from underground plant parts appears to provide the necessary nutritive and environmental influence conducive to successful parasitism.

LITERATURE CITED

1. Brown, R. 1946. Biological stimulation in germination. Nature 157:64-69.
2. Brown, R., and M. Edwards. 1944. The germination of the seed of Striga lutea. I. Host influence and the progress of germination. Ann. Botany 8:131-148.
3. Dobbs, C. G., and W. H. Hinson. 1953. A widespread fungistasis in soils. Nature 172:197-199.
4. Garrett, S. D. 1956. Biology of root infecting fungi. Cambridge University Press. 293 p.
5. Hartelius, Vagn. 1946. Antivitamin effect of sulfanilamide of Aspergillus niger. Compt. rend. trav. lab. Carlsberg, Sér. physiol. 24:178-184.
6. Jackson, R. M. 1957. Fungistasis as a factor in the rhizosphere phenomenon. Nature 180:96-97.
7. Kunkel, L. O. 1914. Physical and chemical factors influencing the toxicity of inorganic salts to Monilia sitophila (Mont.) Sacc. Bull. Torrey Bot. Club 41: 265-293.
8. Linford, M. B. 1939. Attractiveness of roots and excised shoot tissues to certain nematodes. Proc. Helminthol. Soc. Wash. D. C. 6:11-18.
9. Lundegardh, H. 1945. Absorption, transport and exudation of inorganic ions by the roots. Arkiv. Botanik 32A (12): 1-139.
10. Nash, Shirley M., T. Christou, and W. C. Snyder. 1961. Existence of Fusarium solani f. phaseoli in soil as chlamydospores. Phytopathology 51:308-312.
11. Noble, R. J. 1924. Studies on the parasitism of Urocystis tritici, the organism causing flag smut of wheat. J. Agr. Research 27:451-490.
12. Partridge, S. M. 1946. Application of the paper partition chromatogram to the qualitative analysis of reducing sugars. Nature 158:270-271.
13. Plaisted, P. H. 1958. Clearing free amino acid solutions of plant extracts for paper chromatography. Contribs. Boyce Thompson Inst. 19:231-244.
14. Porter, C. A., D. Margolis, and Pamela Sharp. 1957. Quantitative determinations of amino acids by paper chromatography. Contribs. Boyce Thompson Inst. 18: 465-476.
15. Rangaswami, G. 1956. In vitro effect of mycothricin on plant pathogenic bacteria and fungi. Mycologia 48:800-804.
16. Ringel, S. M., and E. S. Beneke. 1956. The influence of certain sugars on the antifungal activity of sodium pyridinethione. Mycologia 48:329-336.
17. Roberts, Edith A. 1916. The epidermal cells of roots. Bot. Gaz. 62:488-506.
18. Strecker, B. 1957. Untersuchungen über die Einwerkung von organischen Fungiziden auf Bodenpilze. Z. Pflanzenkrankh. u. Pflanzenschutz. 64:9-35.
19. Tatum, E. L., and A. C. Giese. 1946. Sulfanilamide and respiration of Neurospora. Arch. Biochem. 9: 15-23.
20. Toussoun, T. A., Shirley M. Nash, and W. C. Snyder. 1960. The effect of nitrogen sources and glucose on the pathogenesis of Fusarium solani f. phaseoli. Phytopathology 50:137-140.
21. Walker, Alma T. 1955. Germination and respiration responses of Myrothecium verrucaria to organic fungicides. Iowa State Coll. J. Sci. 30:229-241.
22. Walker, Alma T., and F. G. Smith. 1952. Effect of Actidione on growth and respiration of Myrothecium verrucaria. Proc. Soc. Expt. Biol. Med. 81:556-559.
23. Wieser, W. 1955. The attractiveness of plants to larvae of root knot nematodes. I. The effect of tomato seedlings and excised roots to Meloidogyne hapla Chitwood. Proc. Helminthol. Soc. Wash. D. C. 22: 106-112.
24. Wieser, W. 1956. The attractiveness of plants to larvae of root-knot nematodes. II. The effect of excised bean, eggplant, and soybean roots on Meloidogyne hapla Chitwood. Proc. Heminthol. Soc. Wash., D. C. 23: 59-64.

STUDIES ON THE BIOLOGY AND PATHOGENICITY OF HEMICYCLIOPHORA ARENARIA

S. D. Van Gundy and R. L. Rackham

Department of Plant Nematology, Paper No. 1270, University of California Citrus Experiment Station, Riverside, California.

The authors thank Charles S. Papp for the illustrations and Dr. H. Schneider for the microtome sections.

Accepted for publication November 25, 1960.

SUMMARY

The sheath nematode (*Hemicycliophora arenaria* Raski) parasitized 10 of 19 host plants tested. Plants in the Cucurbitaceae, Leguminosae, Rutaceae, Solanaceae, and Umbelliferae supported reproduction of the nematode and developed swollen root tips. The highest reproduction occurred on Rutgers tomato. Reduction in growth of citrus and tomato plants ranged from 12% at 25°C to 37% at 30°C. A sandy soil produced the greatest increase in nematode population and reduction in growth as compared with a loamy soil and a 1:1 mixture of the two. The optimum temperatures for reproduction of the nematode on tomato were 30° and 32.5°C. The formation of swellings on Rough lemon roots was due to hyperplasia of the cells surrounding the feeding site of the nematode.

INTRODUCTION.—The sheath nematode (*Hemicycliophora arenaria* Raski 1958) was found parasitizing Rough lemon nursery stock in the Coachella Valley of southern California (1, 2) where it appears to be confined to one ranch. The nematode hangs tightly to freshly dug roots, can be seen with a hand lens, and feeds on the surface of the root tips. The feeding causes a stimulation in cell division resulting in gall formation of the root tips (3). Galls caused by sheath nematodes always occur at the root tips and can be distinguished from galls caused by the root-knot nematode, which usually occur along the root. Since *H. arenaria* was the first member of this genus shown to produce distinctive symptoms on plant roots, we studied its pathogenicity.

OBSERVATIONS AND EXPERIMENTAL RESULTS.—*Host range.*—Reproduction of the sheath nematode was tested on citrus rootstocks in use in California and also on other commercial crop plants commonly grown in the desert region. Two replicates of each plant were infested with 150 hand-picked nematodes and maintained at constant soil temperature of 28–30° C for 3 months. The roots were checked for galls. Nematodes from the roots and soil were recovered by washing through a series of screens.

Reproduction and gall formation occurred on 10 of 19 host plants (Table 1). These susceptible hosts were from the Cucurbitaceae, Leguminosae, Rutaceae, Solanaceae, and Umbelliferae. The highest reproduction occurred on tomato plants where nematodes increased from 150 to 1.5 million in 3 months. Nematode reproduction was always associated with swellings on the root tips. Numerous small swellings were formed on tomato, blackeye bean, pepper, and squash. The swellings produced on celery were large multi-branching growths (Fig. 1,A). Citrus was a relatively poor host for this nematode and fortunately only a few of the commercially grown rootstocks were susceptible. A citrus relative, *Severinia buxifolia*, was the best host among the woody plants tested.

Pathogenicity.—Populations of the sheath nematode were maintained by infesting Rough lemon or tomato seedlings grown in sterilized soil with 100 hand-picked, gravid females. These stock populations were maintained at 28–30°C. Nematodes used in the following experiments were taken from stock populations. The pathogenicity of this species was first demonstrated when each of 10 Rough lemon seedlings was infested with 250 hand-picked nematodes; 5 were maintained at 25° and 5 at 30°C for 5 months. Uninfested seedlings were maintained at the same temperatures. The influence of the nematodes on the growth of the plants and the number of nematodes recovered from soil and roots are given in Table 2. Gall production and reduction in growth were greatest at 30°C. The influence of 30°C on gall production and growth of citrus is shown in Fig. 1,B.

After host-range studies had indicated that tomato was a superior host to citrus, tomatoes were included in subsequent tests at 30°C. Six tomato plants were infested, each with a mass collection of 3,000 nematodes, and maintained in constant-temperature water baths for 3 months. During this time, top growth was removed twice, dried, and recorded as dry weight. The total average dry weights and average numbers of nematodes recovered from roots and soil are given in Table 2.

TABLE 1. Host and nonhost plants of *Hemicycliophora arenaria* infested with 150 hand-picked nematodes and maintained at 28–30°C for 3 months

Host	Galls	No. of nematodes per container
Citrus spp. and relatives:		
Severinia buxifolia	+	12,000
Citrus limon (Rough lemon)	+	5,100
C. limon (Dorshapo sweet)	+	1,250
C. aurantifolia (West Indian lime)	+	1,000
C. reticulata (Cleopatra mandrin)	+	500
C. aurantium (Standard sour)	—	0
C. paradisi (Marsh grapefruit)	—	1
C. sinensis (Homosassa)	—	2
Poncirus trifoliata	—	1
P. trifoliata x *C. sinensis* (Troyer citrange)	—	0
Other hosts:		
Lycopersicon esculentum (Rutgers tomato)	+	1,546,000
Vigna sinensis (Blackeye bean)	+	130,000
Capsicum frutescens, var. *grossum* (Pepper)	+	86,000
Apium graveolens (Celery)	+	71,000
Cucurbita moschata (Squash)	+	55,000
Zea mays (Sweet corn)	—	13
Tagetes sp. (Marigold)	—	0
Gossypium hirsutum (Cotton)	—	2
Vitis vinifera (Thompson seedless)	—	0

TABLE 2. Influence of 250 hand-picked sheath nematodes on the linear growth of Rough lemon seedlings and dry weight of tomato plants

Treatment	Soil temperature (°C)	Experiment	Avg. height (in.)	Avg. dry weight (g)	Avg. no. nematodes per container
Uninfested Rough lemon	25	5 mo	21.6		0
Infested Rough lemon	25	5 mo	19.0[a]		10,000
Uninfested Rough lemon	30	5 mo	23.4		0
Infested Rough lemon	30	5 mo	14.8[b]		160,000
Uninfested tomato	30	3 mo		25.8	0
Infested tomato	30	3 mo		17.4[b]	400,000

[a] Significant at 5% level over the uninfested plants.
[b] Significant at 1% level over the uninfested plants.

Along with the obvious symptoms of swellings on the root tips, there was a reduction in the total number of feeder roots. This in turn reduced the linear growth of Rough lemon seedlings by 36% and the dry weight of tomato plants by 28% at 30°C.

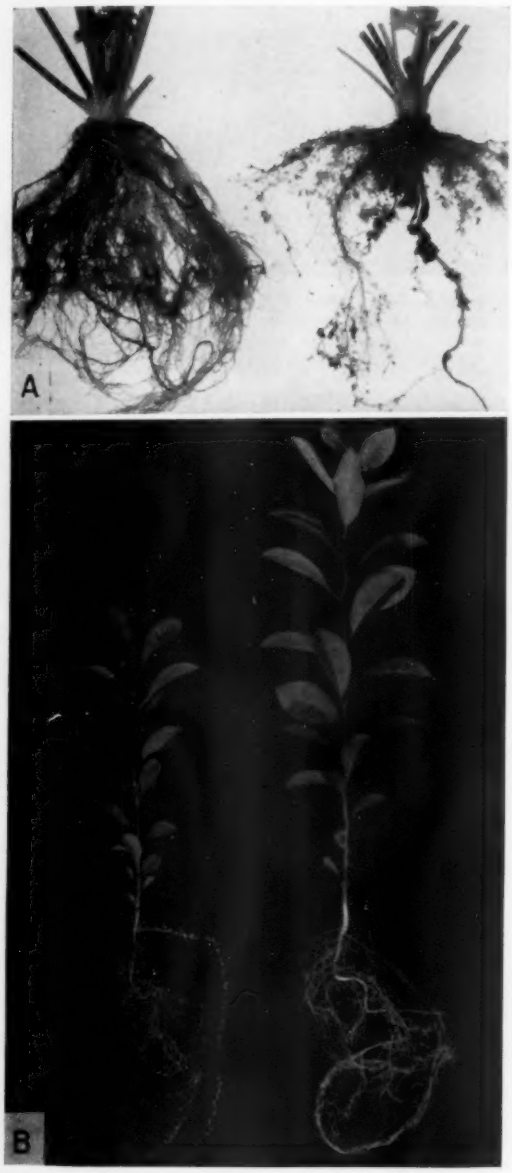


Fig. 1. (A) Left: uninfested celery roots. Right: infested celery roots showing large multi-branched swellings produced by *H. arenaria*. **(B)** Rough lemon seedlings. Left: infested with 250 hand-picked nematodes grown for 5 months at 30°C. Right: uninfested.

Influence of soil temperature.—The optimum constant soil temperature for reproduction of the nematode was found by infesting each of 24 tomato plants with 100 hand-picked nematodes. The plants were grown in groups of 4 in soil temperature tanks for 10 weeks at 20°, 25°, 27.5°, 30°, 32.5°, and 35°C. Dry weights of roots were recorded after nematodes had been collected, and are presented for comparative purposes, with number of nematodes recovered (Fig. 2). The optimum temperature range for reproduction was 30–32.5°C. Almost no reproduction took place at 20°C. Although reproduction was reduced at 35°C, large numbers were still recovered from the soil at this temperature. The optimum temperature for root growth of the infested tomato plants was 27.5°C. Above 27.5°C, root growth decreased as the nematode population increased.

Longevity in soil.—Soil containing approximately 500 nematodes (adults and larvae) per pint—plus any eggs there might have been—was stored in six 1-gal containers, three each at 25° and 30°C. The soil was kept moist and all weeds were removed during the experiment. The containers were sampled at monthly intervals for 6 months; then, tomato seedlings were planted and grown for 2 months at 30°C. The seedlings were removed and examined for galling and presence of nematodes.

During the first month, the population at both temperatures fell sharply from 500 nematodes per 50 cc of soil to 50 nematodes per 50 cc. The population stabilized after the second month at approximately 25 nematodes per 50 cc and remained at that level for 4 months. The number of nematodes surviving storage at 30° decreased at each sampling. A few of the nematodes survived the 6-month period of storage at each temperature and were capable of producing large populations after 2 months in the presence of a host plant. The large loss in nematode population during the first month was due to the poor survival of all

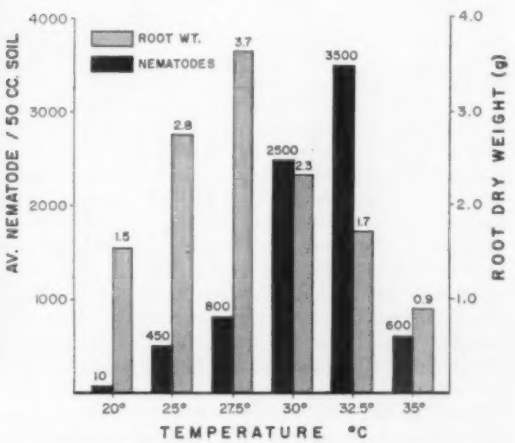


Fig. 2. Influence of temperature on reproduction of *H. arenaria* and resulting dry weights of host tomato roots.

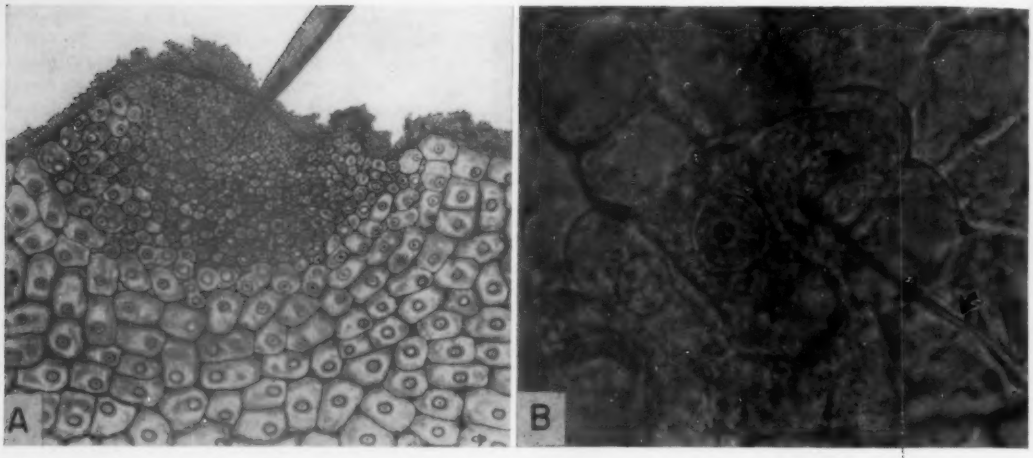


Fig. 3. (A) Free-hand drawing of a nematode feeding site from a cross section of a Rough lemon root swelling, showing the position of stylet in relation to the host tissue and response of the host tissue to the feeding stimulus. **(B)** Photomicrograph showing stylet, dividing cells, and enlarged nuclei.

the larval stages. Only the adults were able to survive long periods without food. This is in agreement with earlier observations made on storage life in petri dishes and water (4).

Influence of soil texture.—The soil at the location where this nematode was originally found was 75% sand, 9% silt, and 16% clay. The first attempt to culture this nematode in a greenhouse mix of 46% sand, 37% silt, and 17% clay was relatively unsuccessful. A soil consisting of approximately 90% sand, 5% silt, and 5% clay was then used for 2 of the following treatments: 100% of the sandy soil, 100% of the above greenhouse mix, and 1:1 mixture of the two soils. Each container was infested with 10,000 nematodes. Tomato plants were used as the host and were grown for 5 months. Tops were harvested 3 times during this period. Total numbers of nematodes recovered from the containers and total dry weights are given in Table 3. Greatest reduction in growth of the tomato plants and greatest reproduction of the nematode occurred in the sandy soil. There was no significant change in the nematode population and no significant reduction in growth of the tomato plants in the greenhouse soil. The mixture was intermediate.

Histopathology.—Large root galls from Rough lemon seedlings, with numerous nematodes attached, were fixed with Randolph's Modified Navashin Fluid and sectioned with a freezing microtone into 40 μ and 60 μ sections. These sections were stained in Orange G lactophenol. The areas in the immediate vicinity of the nematode stylet appeared to be undergoing cell division, giving a dense pattern of small cells, as compared with the larger cells surrounding the area. The nuclei were more deeply stained and larger than those in adjacent areas. The stylet was usually imbedded 6–8 cells deep in the cortex. A free-hand drawing made from photomicrographs and observations of a feeding site is presented in Fig. 3,A. The photomicrograph in Fig. 3,B shows the inserted stylet near dividing cells which contain hypertrophied nuclei.

Observations of nematodes feeding on tomato roots on water agar in petri dishes indicate that feeding initially inhibits cell elongation, evidently because of a lack of cell differentiation and maturation. Then hyperplasia of the cortex in the feeding sites forms the root swellings. If the nematodes are removed, the swelling stops and one or more branch roots originate from the swelling.

Discussion.—Although this nematode is at present restricted in its known distribution, it is potentially an important economic plant parasite. Under optimum conditions of temperature and soil texture, large populations of this nematode build up in fairly short periods of time. A wide variety of hosts may be attacked, and growth may be reduced, as in the hosts studied, by as much as 35%. Immature stages of this nematode perished during fallow periods; adults, however, were able to survive for at least 6 months at a constant temperature of 30°C.

Table 3. Influence of soil texture on the reduction in growth of tomato plants and on the increase in nematode populations

Soil treatments	Avg. dry weight (g)		Avg. no. nematodes per container
	Uninfested	Infested	
100% sandy soil	32.4	21.7[b]	700,000[b]
50% sand-50% topsoil	33.9	28.9[a]	50,000[b]
100% topsoil	31.5	30.6	10,000

[a] Significant at 5% level.
[b] Significant at 1% level.

The observations and studies on the feeding habits of *H. arenaria* and on the histopathology of the host indicate a physiological stimulus is produced by the nematode. The stimulus alters the host in the following way to produce a substrate necessary for the nematode: Initial feeding by a few nematodes (larvae or adults) almost immediately inhibits the process of cell elongation at some distance from the feeding site. Cell division continues in the apical meristem (feeding site), probably at an accelerated rate. The nuclei are prominent and somewhat hypertrophied. This process continues as long as the nematodes feed. Removal of the nematodes allows the return of apparently normal root growth. Finally, among the host plants tested, gall production always occurred on the hosts that supported reproduction of the nematode, and only on those hosts. This is in contrast to *Meloidogyne* spp. which may produce galls on host plants that are not capable of supporting reproduction.

LITERATURE CITED

1. Raski, D. J. 1958. Four new species of Hemicycliophora de Man, 1921, with further observations on H. brevis Thorne, 1955 (Nematoda: Criconematidae). Proc. Helminthol. Soc. Wash. 25:125-131.
2. Van Gundy, S. D. 1957. The first report of a species of Hemicycliophora attacking citrus roots. Pant Disease Reptr. 41:1016-1018.
3. Van Gundy, S. D. 1958. The pathogenicity of Hemicycliophora arenaria on citrus. (Abstr.) Phytopathology 48:399.
4. Van Gundy, S. D. 1959. The life history of Hemicycliophora arenaria Raski (Nematoda: Criconematidae). Proc. Helminthol. Soc. Wash. 26:67-72.

WHERE CABBAGE APHIDS CARRY TRANSMISSIBLE VIRUS B

Gert B. Orlob and R. H. E. Bradley

Respectively, National Research Council Postdoctorate Fellow, Department of Biology, University of New Brunswick, Fredericton, N. B.; and Research Officer, Entomology and Plant Pathology Section, CDA Research Station, Fredericton.

Contribution No. 42. CDA Research Station, Fredericton.
The work was supported in part by the National Research Council of Canada.
Accepted for publication November 26, 1960.

SUMMARY

It was recently postulated that the cabbage aphid (*Brevicoryne brassicae* L.) carries transmissible cabbage virus B on its stylets after short acquisition feedings on an infected plant, but carries the virus elsewhere after some hours on the source. We obtained no evidence that *B. brassicae* carries transmissible virus B other than on its stylets. When aphids fed for some hours on infected plants and their stylets were treated with dilute formalin or irradiated with ultraviolet light, all but occasional ones ceased to transmit virus B. By contrast, the same treatments did not seem to affect the aphid transmission of 3 viruses that persist in their vectors; barley yellow dwarf virus, sugar beet yellow net virus, and turnip latent virus.

Introduction.—The cabbage aphid (*Brevicoryne brassicae* L.) seems to transmit cabbage virus B in a way that is not characteristic of most viruses that do not persist for long in their vectors. Some of the uncharacteristic features reported are: 1) pre-acquisition fasting has little or no effect on transmission (6, 7); 2) prolonging acquisition feeding time increases the level of transmission (5, 6, 7), and 3) *B. brassicae* retains virus B for some hours after leaving infected plants (5, 6, 7). Chalfant (5) recently reported a bimodal curve of transmission efficiency based on different acquisition feeding times. A summary curve of his experiments showed peaks of maximum transmission after acquisition feeding times of 5 minutes and 8 hours, and a trough at 15 minutes. He presented evidence that the cabbage aphid transmits virus B nonpersistently after short probes on the source plant, during which the virus was carried by the stylets. The virus was transmitted semipersistently, however, after aphids had fed some hours on source plants and the virus was then located elsewhere, presumably internally. The main evidence for this was that when aphids had probed the source briefly, formalin treatment of the stylets caused them to cease transmitting. After they had spent some hours on the source, however, formalin treatment of stylets did not cause them to cease transmitting.

Since Chalfant's was the first claim that an aphid

transmits one virus in two ways, we decided to study it further. Presumably, *B. brassicae* transmits virus B via its stylets after brief probes (2, 5); we therefore confined our studies to the newly postulated semipersistent part of the bimodal transmission pattern.

MATERIALS AND METHODS.—The strain of cabbage virus B used, obtained from R. J. Shepherd, Madison, Wis., is supposed to have been derived from the strain used by Chalfant (5). Also used were 3 viruses that persist in their aphid vectors: beet yellow net virus, from E. S. Sylvester, Berkeley, Calif.; turnip latent virus, from J. P. MacKinnon, Fredericton, N. B.; and barley yellow dwarf virus, from a field-infected plant near Fredericton. The viruses, the vectors, and the plants used as both source and test plants were: cabbage virus B, *Brevicoryne brassicae* (L.), and *Brassicae oleracea* (cauliflower); barley yellow dwarf, *Rhopalosiphum fitchii-padi* (complex), and *Hordeum vulgare* (barley); beet yellow net, *Myzus persicae* (Sulz.), and *Beta vulgaris* (sugar beet); and turnip latent, *Myzus persicae* (Sulz.), and *Physalis floridana*.

In experiments with virus B, all aphids fed on infected plants overnight; with the other viruses, they fed on the source for several days. Since most aphids had their stylets inserted some distance into the source plants after all feeding times, they were anesthetized *in situ*, after which the labium could be manipulated to expose more or less of the stylets as needed. The method of doing this, as well as that of treating the stylets with formalin or ultraviolet radiations, has been described (3, 4). In tests with formalin, the stylets were inserted for 30 seconds into a capillary tube filled with 0.25% or 1.0% formalin. As controls, other aphids were treated the same way but with water. Aphids exposed to ultraviolet radiations were held for 1 minute in front of a mercury-vapor discharge lamp at a distance where the intensity of irradiation of 2537 Å was about 10 mw per cm^2. Each treated aphid was allowed to feed for a day or more on a young healthy test plant to learn if it would transmit virus.

RESULTS.—When *B. brassicae* fed overnight on plants infected with cabbage virus B, formalin treatment of the distal ends of the stylets caused all but one aphid to cease transmitting virus, whereas 40% of the aphids transmitted the virus after their stylets were treated with water (Table 1). In contrast, formalin treatment of the stylets of aphids carrying a persistent virus did not reduce the numbers of transmissions (Table 1). In all four series of experiments, randomly selected aphids were tested for infectivity without any treatment of their mouthparts. The transmission results for these aphids have been omitted because they were only slightly higher than those of aphids whose stylets were treated with water. This slight difference was probably due to the fact that anaesthetizing aphids and manipulating their mouthparts makes them reluctant to feed for awhile after recovery, and possibly injures an occasional aphid.

Table 2 summarizes the results of experiments in which aphids were irradiated with ultraviolet light. In one series, cabbage aphids did not transmit virus B if the tip 15 μ of their stylets or more were exposed to the radiations, but did so if irradiated with their stylets ensheathed in the labium. In similar tests with barley yellow dwarf virus, which persists in its vector, exposing the stylet tips to ultraviolet radiations did not seem to affect transmission.

TABLE 1. Effect of treating the stylet tips of aphids with dilute formalin on the transmission of previously acquired virus

	Tip of stylets inserted 30 seconds in		
		Formalin	
Virus	Water	0.25%	1.0%
Cabbage B	16/40[a]	0/40	1/40
Turnip latent	8/37		9/38
Barley yellow dwarf	10/17		9/17
Beet yellow net	8/15		10/13

[a] Number of test plants infected/number colonized with 1 aphid each.

TABLE 2. Effect of exposing aphid stylets to ultraviolet radiations on the transmission of previously acquired virus

	Distance stylets bared (μ)	Transmission	
Treatment		Cabbage virus B	Barley yellow dwarf virus
Not irradiated	15	22/48[a]	10/19
Irradiated 1 min.	0	15/48	
	15	1/48	
	>15	0/48	8/19

[a] Number of test plants infected/number colonized with 1 aphid each.

DISCUSSION.—When we treated the stylets of *B. brassicae* with formalin or exposed them to ultraviolet radiations, the effect on transmission of cabbage virus B was similar to that obtained earlier with potato virus Y (3, 4) and other viruses that do not persist in their aphid vectors (2, 8). On the assumption that these two treatments would inactivate virus carried by the stylets, we conclude that cabbage virus B is yet another virus that aphids carry *on* or *within* their stylets even after they have fed for some time on infected plants.

In contrast to carrying virus on their stylets, aphids also transmit viruses that are believed to pass internally through the vector. Once aphids acquire these viruses, they are able to transmit them for days and often for life. In our tests with 3 viruses that persist in their vectors, treating the stylets in ways assumed to inactivate virus did not affect transmission, further evidence that such viruses are carried internally. It also shows that these treatments did not affect the feeding behavior in ways that prevented transmission.

A point that calls for comment is why our results differ from those of Chalfant (5). At first we treated the stylets with 1% formalin, whereas Chalfant used 0.25%; thus, it seemed possible that stronger formalin was needed to inactivate virus B on the stylets after long acquisition feedings. Nevertheless, subsequent tests with both 0.25% and 1.0% formalin showed no

difference in transmission. Our source of cabbage virus B appears the same as that used by Chalfant (5), but our strain of *B. brassicae* was not. Different strains of aphids have been shown to vary in the efficiency with which they transmit certain viruses (1). It is therefore possible—but seems improbable—that our strain of *B. brassicae* transmits virus B by one mechanism whereas that used by Chalfant by two, as he postulated. Why our results differ from those of Chalfant remains unsolved.

LITERATURE CITED

1. Bjorling, K., and F. Ossiannilsson. 1958. Investigations on individual variations in the virus-transmitting ability of different aphid species. Socker Handlingar 14:1-13.
2. Bradley, R. H. E. and R. Y. Ganong. 1957. Three more viruses borne at the stylet tips of Myzus persicae (Sulz.). Can. J. Microbiol. 3:669-670.
3. Bradley, R. H. E., and R. Y. Ganong. 1955. Some effects of formalin on potato virus Y in vitro and ability of aphids to transmit the virus when their stylets were treated with formaldehyde. Can. J. Microbiol. 1:783-793.
4. Bradley, R. H. E., and R. Y. Ganong. 1955. Evidence that potato virus Y is carried near the tip of the stylets of the aphid vector Myzus persicae (Sulz.) Can. J. Microbiol. 1:775-782.
5. Chalfant, R. B. 1959. Transmission of cabbage virus A and B by the cabbage and green peach aphid. Ph.D. thesis, University of Wisconsin. 187 p.
6. Hamyln, B. M. G. 1955. Aphid transmission of cauliflower mosaic on turnips. Plant Pathol. 4:13-16.
7. Hoof, H. A. van. 1954. Differences in the transmission of the cauliflower mosaic virus by Myzus persicae (Sulz.) and Brevicoryne brassicae (L.) Tijdschr. Plantenziekten 60:267-272.
8. Orlob, G. B., and R. H. E. Bradley. 1960. Drei weitere Blattlausarten, die das Y-Virus des Kartoffel mit den Stechborstenspitzen übertragen. Z. Pflanzenkrankh. u. Pflanzenschutz 67:407-409.

ADAPTATION OF SCLEROTINIA FRUCTICOLA AND SCLEROTINIA LAXA TO HIGHER CONCENTRATIONS OF FUNGICIDES

Rajendra K. Grover and J. Duain Moore

Respectively, research assistant and Professor of Plant Pathology, Department of Plant Pathology, University of Wisconsin, Madison. Portion of a Ph.D. thesis by the senior author, University of Wisconsin.
Accepted for publication December 1, 1960.

SUMMARY

Continuous exposure of an isolate of *Sclerotinia fructicola* and of *S. laxa* to Acti-dione, dodine, ferbam, Phaltan, and dichlone at concentrations higher than the levels toxic to these fungi resulted in development of a temporary resistance against some. The *S. fructicola* isolate grew on the higher concentrations of Acti-dione, dodine, and Phaltan; and the *S. laxa* isolate grew on higher concentrations of Acti-dione and Phaltan only. The adaptation process was coupled with some morphological changes in colony characters. Adaptation was specific for the respective fungicides, and did not involve genetic selection, since the adapted isolates eventually reverted to the parent types after growing for 2-5 generations on media without any fungicide.

Introduction.—Adaptation or development of resistant strains of bacteria, particularly when treated with antibiotics, is reported in the literature. Fungi, in contrast, seem not to react to fungicides so spectacularly even though some chemicals have been used against fungi in the field for many years. Taylor (8) presented evidence for the development of resistant strains of spores of *Physalospora obtusa* (Schw.) Cke. in orchards where copper fungicides were used continuously. Mader and Schneider (4) studied the tolerance to copper sulfate of monoascosporic isolates of *Sclerotinia fructicola* (Wint.) Rehm. and found that cultures could be trained to tolerate high concentrations of the chemical. Parry and Wood (5, 6, 7) recently studied the adaptation of *Botrytis cinerea* Deb. to organic and inorganic chemicals and reported the development of genetically resistant strains of the fungus that grew at very high concentrations of the chemicals used. They were unable to produce similar resistant strains of *Venturia inaequalis* (Cke.) Wint. by the same methods. Horsfall (3) considered that, with the use of newer chemicals, which are rather selective and more specific in their actions than many of the older fungicides, resistant strains of fungi are likely to appear more frequently and become of greater practical importance. Because of this, the possibility was investigated of adapting two brown-rot-inciting organisms—*S. fructicola* and *S. laxa*

TABLE 1. Adaptation of *Sclerotinia fructicola* to increased concentrations of Acti-dione, dodine, and Phaltan

Test chemical	Inoculum from cultures grown at (μg/ml)	Period of growth (weeks)	Maximum concentration (μg/ml) allowing growth from inoculum
Acti-dione	0.0	0	8.0
	8.0	2	10.0
	10.0	2	15.0
	15.0	3	17.5
	17.5	3	20.0
	20.0	4	22.5
Dodine	0.0	0	20.0
	20.0	2	24.0
	24.0	2	30.0
	30.0	4	35.0
	35.0	4	40.0
	40.0	4	42.5
	42.5	4	45.0
Phaltan	0.0	0	20.0
	20.0	2	25.0
	25.0	4	32.5
	32.5	3	37.5
	37.5	4	50.0
	50.0	4	60.0
	60.0	4	62.5

Aderh. & Ruhl.—to grow at high concentrations of some fungicides that have been useful in controlling brown rot in the field. The magnitude of the adaptations of the two fungi also was studied.

MATERIALS AND METHODS.—The isolate of *S. fructicola* was obtained from infected sour cherry fruits of the Early Richmond variety, and that of *S. laxa* was obtained from infected spurs of sour cherry variety Montmorency—both from Door County, Wis. Subcultures from single conidial isolates were used in all the studies. The fungicides used were: Acti-dione—chemically pure β-[2-(3,5-dimethyl-2-oxycyclohexyl)-2-hydroxyethyl]-glutarimide (Cycloheximide); dodine—chemically pure *n*-dodecylguanidine acetate; Fermate—76% ferric dimethyldithiocarbamate (ferbam); Phaltan—50% *n*-trichloromethylthiophalimide; and, Phygon XL—50% 2,3-dichloro-1,4-naphthoquinone (Dichlone).

Trommer's malt-extract agar medium (Trommer's extract of malt, 25 g; agar, 17 g; and distilled water to make 1 liter) was prepared at twice the final concentration, dispensed in 50-ml portions in pharmaceutical bottles, and sterilized. Stock solutions of the fungicides were freshly prepared by dissolving the chemicals in sterile distilled water or in 25% ethanol, if insoluble in water. Stock solutions were pipetted into proper volumes of sterile distilled water in 100-ml Erlenmeyer flasks to give 50 ml of the fungicide at twice the final desired concentration. The diluted fungicides were added to partially cooled medium (about 45°C) in the pharmaceutical bottles and shaken well; 25 ml of the mixture were poured into each of 4 petri dishes and allowed to gel. Several concentrations of each fungicide were used for each treatment. Four discs (about 5 mm diameter) of inoculum from 5- to 7-day-old cultures growing on malt extract agar medium were placed on each petri dish, and 4 replicates were used for each treatment with each fungus. The inoculated petri dishes were incubated 2-4 weeks at 24-26°C. When mycelial growth appeared from one or more discs on the medium containing increased concentrations of the fungicide, subcultures from this growth were made on media containing progressively higher concentrations of the same fungicide. This process was repeated until a concentration was reached on which no growth was obtained. The pH of the medium was maintained at 6.0-6.5 in all cases.

RESULTS.—*Sclerotinia fructicola*.—The process of adaptation was begun at fungicide concentrations lower than those required to inhibit mycelial growth of the fungus as determined earlier (2). Since mycelial inhibition had been obtained with Acti-dione at 10 μg/ml, the adaptation process for this chemical was begun at 8μg/ml. After being grown for 2 weeks at this concentration, subcultures were made on media containing 10, 12, 14, and 16 μg/ml of Acti-dione. Growth was obtained only on medium containing 10 μg/ml. Subcultures were then made on media containing progressively higher concentrations of Acti-dione. This process was continued until no growth was obtained at a concentration higher than 22.5 μg/ml of Acti-dione even after 4 weeks incubation or repeated subculturing at 22 μg/ml.

Similar treatment was given with other fungicides, but evidence of adaptation was obtained only with dodine and Phaltan, in addition to Acti-dione. The concentrations of dodine and Phaltan required to inhibit mycelial growth of this organism had been, respectively, 25 and 24 μg/ml (2); the adaptation process for these fungicides was begun at 20 μg/ml. The isolate was adapted to grow up to a concentration of 45 μg/ml of dodine, and 62.5 μg/ml Phaltan. The results are presented in Table 1.

TABLE 2. Adaptation of *Sclerotinia laxa* to increased concentrations of Acti-dione and Phaltan

Test chemical	Inoculum from cultures grown at (μg/ml)	Period of growth (weeks)	Maximum concentration (μg/ml) allowing growth from inoculum
Acti-dione	0.00	0	0.03
	0.03	2	0.06
	0.06	2	0.10
	0.10	3	0.17
	0.17	3	0.50
	0.50	4	0.75
	0.75	4	1.00
	1.00	4	1.25
Phaltan	0.00	0	20.00
	20.00	2	26.00
	26.00	2	32.00
	32.00	3	42.50
	42.50	3	47.50
	47.50	4	52.50
	52.50	4	55.00

Sclerotinia laxa.—By the same procedure, the *S. laxa* isolate was adapted to grow on media containing increased concentrations of only Acti-dione and Phaltan—raising the respective concentrations for inhibition of mycelial growth from 0.05 and 24 μg/ml (2) to 1.25 μg/ml and 55 μg/ml. The results are presented in Table 2.

Colony characteristics of the adapted isolates.—The adapted isolates of the 2 fungi grew very slowly, and the mycelium formed a dense fringe on the agar surface. This fringe produced a darkly pigmented, slightly convoluted, mycelial crust with a texture similar to that of a young sclerotium. Growth was entirely superficial, and very few hyphae penetrated the agar. The mycelial pad could be readily detached from the medium. The characteristic lobe formation of *S. laxa* was lacking, though the margins of the colonies remained irregular. No sporulation was seen in any of the adapted cultures.

Magnitude of adaptation.—The adapted isolates of *S. fructicola* and *S. laxa* differed markedly from the parent isolates, especially in colony character and toleration to specific fungicides. An isolate adapted to grow at increased concentrations of Acti-dione did not grow on media containing increased concentrations of Phaltan, and vice-versa. An isolate adapted to grow at increased fungicide concentrations grew much faster at slightly lower concentration of the same fungicide than the isolate previously adapted only at this concentration. The adapted isolates, when grown on normal media without any fungicide, soon reverted to parental type, gradually losing their capacity to grow at increased concentrations. After 1 transfer on media with no fungicides, adapted isolates could be grown at increased concentrations of the respective fungicide only after a long period of incubation. The adapted isolate of *S. fructicola* usually reverted to the parental type after 2-3 generations on media with no fungicide, whereas it took 5-6 transfers before the *S. laxa* isolate reverted to the parent type. Loss of adaptation, however, was gradual in both fungi.

Discussion.—The property of selective adaptation of the *S. fructicola* isolate to grow at increased concentrations of Acti-dione, dodine, and Phaltan; and of the *S. laxa* isolate to grow on increased concentrations of Acti-dione and Phaltan only, was borne out in the present investigations. Since the adapted isolates reverted to the parental types after growing for 2-5 generations on media with no fungicide, the phenomenon seemed purely temporary resistance rather than genetic selection. Parry and Wood, (5, 6, 7) in studies on adaptation of *Botrytis cinerea* to organic and inorganic chemicals, reported a genetic selection in the resistant strains that grew on increased concentrations of the chemicals. Those selected strains, they pointed out, were able to transmit their resistance through conidia or mycelium for a few generations, but nevertheless reverted to parental types. In the present studies, the adapted isolates could grow at a lowered concentration of the specific fungicides for only 1 generation without losing their ability to grow at the higher concentration. Buxton (1), in a recent review on adaptation in microorganisms, suggests that neither mutation nor enzymic adaptation theories completely explain various kinds of adaptations. Parry and Wood (7) suggested that resistance to fungicides was built up by fungi gradually if inoculum did not come in close proximity to media containing higher concentrations of fungicides. Lack of proximity allowed the mycelium to grow and subsequently become adapted to higher concentrations of fungicides. The present studies indicate that when the inoculum disc was put on medium containing an increased concentration of fungicide, the medium of the initial inoculum provided a base for new mycelial growth.

Freshly grown mycelium from the inoculum was well enough adapted to withstand a certain increase in fungicide concentration in the medium. As pointed out by Parry and Wood (7), increased resistance of the hyphae depended on a reduced permeability of the cell wall to the fungicides. This might also be associated with a reduction in permeability to essential nutrients, resulting in poor growth of the organism. Slow growth of adapted isolates was a consistent feature in the present studies. The possibility of production of adaptive enzymes during the process of adaptation is more likely than selection of resistant mutants. Since the 2 fungi did not sporulate at increased fungicide concentrations, the probability of the successful establishment of temporary mutants is remote.

LITERATURE CITED

1. Buxton, E. W. 1960. Heterokaryosis, saltation, and adaptation. *In* Plant pathology—an advanced treatise, ed. J. G. Horsfall and A. E. Dimond. Vol. 2:359-405. Academic Press, New York.
2. Grover, R. K. 1960. Laboratory and field evaluations of antibiotics and synthetic organic fungicides used for the control of Sclerotinia fructicola (Wint.) Rehm. and Sclerotinia laxa Ader. & Ruhl. on cherries. Ph.D. thesis, Univ. of Wisconsin, Madison, Wisconsin. 107 p.
3. Horsfall, J. G. 1956. Principles of fungicidal action. Chronica Botanical Co., Waltham, Massachusetts. 279 p.
4. Mader, E. O., and C. L. Schneider. 1948. Changes induced in Sclerotinia fructicola on copper sulfate media. (Abstr.) Phytopathology 38:17.
5. Parry, K. E., and R. K. S. Wood. 1958. The adaptation of fungi to fungicides: adaptation to copper and mercury salts. Ann. Appl. Biol. 46:446-456.
6. Parry, K. E., and R. K. S. Wood. 1959. The adaptation of fungi to fungicides: adaptation to captan. Ann. Appl. Biol. 47:1-9.
7. Parry, K. E., and R. K. S. Wood. 1959. The adaptation of fungi to fungicides: adaptation to thiram, ziram, ferbam, nabam, and zineb. Ann. Appl. Biol. 47:10-16.
8. Taylor, J. 1953. The effect of continual use of certain fungicides on Physalospora obtusa. Phytopathology 43:268-270.

A MOSAIC DISEASE OF HIBBERTIA VOLUBILIS

Robert M. Endo

Department of Plant Pathology, University of California, Los Angeles.

The author thanks K. F. Baker for suggesting this problem, and K. F. Baker and J. G. Bald for advice and suggestions given throughout this study.

Accepted for publication December 2, 1960.

SUMMARY

A mosaic disease of *Hibbertia volubilis*, of widespread occurrence in the coastal areas of California, was found to be caused by strains of the common cucumber mosaic virus (CMV). The virus was mechanically transmitted with difficulty from 8 *Hibbertia* clones to 13 host species; and with ease from 8 sub-inoculated *Nicotiana glutinosa* plants. Percentage of transmission from *Hibbertia* to test plants was doubled by growing diseased source plants at 60°F for 4 weeks. Although symptoms on the 13 hosts were uniformly mild, they compared well with symptoms induced in these hosts by 3 known strains of common CMV, and with descriptions in the literature. Physical properties of the 8 isolates were identical to those of 2 strains of CMV and to those reported for CMV: inactivation in 10 minutes at 70°C, a dilution end point of 1:100,000 and aging *in vitro* at 5 days. Each of the 2 CMV isolates protected against local lesion formation on cowpea by each of the 8 *Hibbertia* isolates, and each of the 8 *Hibbertia* isolates protected against the CMV1 strain. Symptoms on *Hibbertia* clones 1 and 3 were very severe at a constant temperature of 10° and 15°C, severe at 20°C, moderate at 23.3°, and mild at 26.7°C. Some virus-free terminal shoots were produced when mosaic-affected *Hibbertia* plants were grown at temperatures that averaged 28.3°C; when temperatures averaged 32.8°C, all terminal shoots were free of virus. The virus was not inactivated in the diseased plants at either temperature. In limited tests, the virus was not readily transmitted from diseased plants to healthy cuttings with a cutting knife.

INTRODUCTION.—In California, the Guinea Gold vine, *Hibbertia volubilis* Andr. (*H. scandens* (Wild) Gilg.), is affected with mosaic symptoms so commonly that not a single healthy plant has been observed either in home plantings or in nurseries. The Guinea Gold vine is native to Queensland and New South Wales, where it grows as a prostrate or twining shrub with solitary yellow flowers. In California it is grown primarily in the cool coastal areas where it fails to produce seeds; thus clonal propagation is necessary and is suspected as being the major factor responsible for the high incidence of mosaic-affected plants.

Inoculations from *Hibbertia* showed that a virus was present in affected plants. This paper reports studies on symptoms, host range, transmission, physical properties, temperature relations, and a method of obtaining virus-free plants. A preliminary account has been published (1).

MATERIALS AND METHODS.—Eight clones of *H. volubilis* affected with mosaic symptoms of varying types and degrees of severity were studied. The viruses recovered from the 8 clones are referred to as isolates 1-8, and the respective plants as clones 1-8. Six of the 8 isolates were maintained in the 6 original *Hibbertia* clones, and no special methods of purification were used. During the study, 6 clones showed no evidence of being infected with a mixture of strains, but inoculum from clones 5 and 6 caused development of a bright yellow mosaic in occasional test plants instead of the usual yellowish-green mottle. These 8 clones, and 6 others which were studied less intensively, were obtained from various nurseries and home plantings in southern California. Plants from 6 of the 8 clones were used in the temperature experiments; and, when freed of virus, in experiments involving transmission from mosaic-affected *Hibbertia* to virus-free *Hibbertia*. *Nicotiana glutinosa* L. was usually used as the source plant because of low transmission from *Hibbertia*.

Mechanical inoculations were made by grinding infected leaves with a mortar and pestle, and diluting the extract with equal parts of McIlvaine's phosphate-citrate buffer (pH 7). The extract was rubbed lightly over leaves of test plants that had been previously dusted with celite or Carborundum powder and the leaves washed with tap water.

Plants were grown in a steam-sterilized sand-peat mix, and were fertilized weekly with a liquid mixture containing both macro- and micro-elements. Inoculations were usually made when plants were in the cotyledon or the first or second true-leaf stage; 5-30 plants were used per test. Recovery inoculations were made to suitable indicator plants, usually *N. glutinosa* L., *Spinacia oleracea* L., or *Chenopodium amaranticolor* L.

Field and greenhouse symptoms.—Naturally infected *Hibbertia* plants manifested mild to severe leaf mosaic, leaf deformation, stunting, and usually, considerable leaf chlorosis or yellowing. Leaf deformation occurred as a change in shape, margins, and in the surface, which usually became rough and irregular, due to raised veins and sunken interveinal tissue. Flower set and flower size also appeared to be reduced.

Symptoms were severe, persistent, and completely systemic during the cooler months of the year; during the warmer months, symptoms tended to be mild

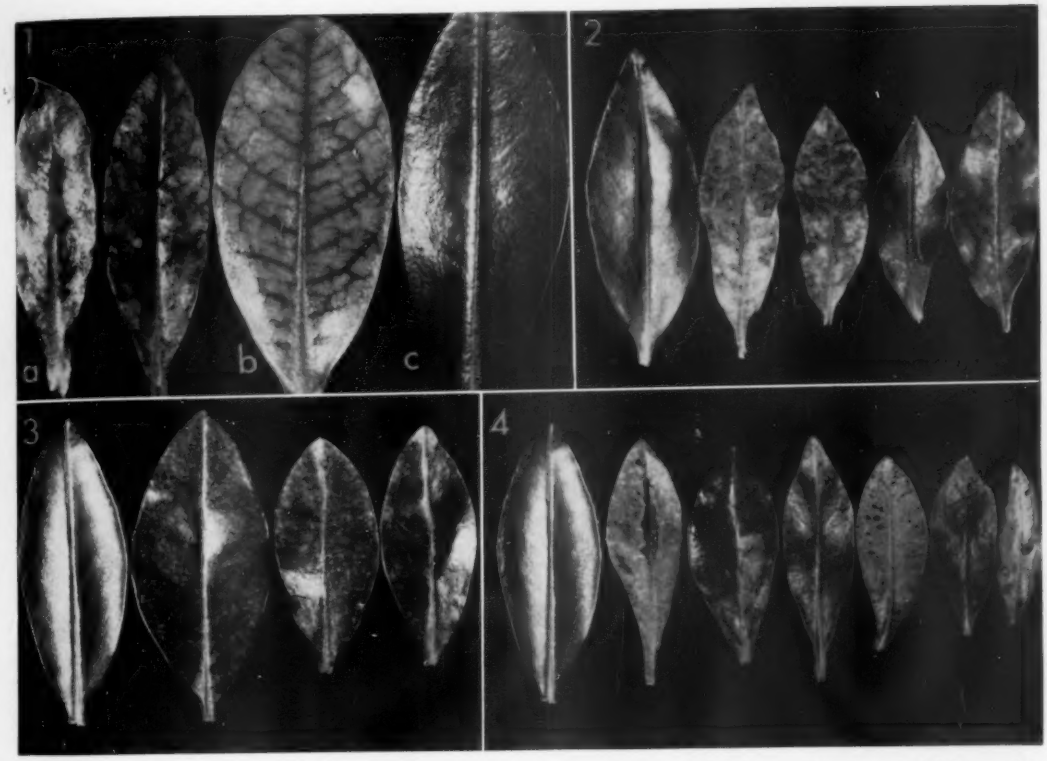


Fig. 1-4. **1)** Leaves of *H. volubilis* affected with (a) mosaic and mineral deficiency, (b) mineral deficiency, (c) healthy leaf. **2)** Symptoms caused by cucumber mosaic virus isolates 1, 3, 4, 7, and 8. **3)** Symptoms caused by isolate 2. **4)** Symptoms caused by isolates 5 and 6.

and usually incompletely systemic, particularly following periods of rapid growth. Although considerable seasonal recovery occurs, observations indicate that the effects of the disease are cumulative and that old plantings are considerably reduced in size and vigor.

The initial systemic symptoms on inoculated plants in the greenhouse were irregular yellow-green blotches that first appeared on young foliage. The spots, which varied from 0.5 mm-2.0 cm, were mostly circular, and frequently were delimited by the veins. This was followed by the general mosaic, stunting, and deformation of leaves that characterize the disease in the field. Notably absent, however, were the conspicuous leaf yellowing and chlorosis that are frequently associated with the disease in the field. It was learned early in these studies that *Hibbertia* suffers readily from mineral deficiency, resulting in a marked chlorosis and yellowing of the interveinal areas (Fig.1). Frequent application of fertilizers in the greenhouse has almost completely eliminated this condition in healthy and diseased *Hibbertia*.

Isolates 1, 3, 4, 7, and 8 caused a mild greenish-yellow mosaic (Fig. 2). Isolate 2 produced a bright yellow mosaic (Fig. 3) and isolates 5 and 6 (Fig. 4) considerable leaf distortion and a strong mosaic mottle, consisting of small islands of dark green tissue and large areas of greenish yellow. All isolates occasionally produced oak leaf and ring patterns on old leaves.

Mechanical transmission.—Since *Hibbertia* plants free of mosaic symptoms were not available initially, sap-transmission was attempted from the 8 mosaic-affected clones to plants of *N. glutinosa*, *N. tabacum*, *Capsicum annuum*, and *Lycopersicon esculentum*. Virus was transmitted from all the mosaic-affected *Hibbertia* clones; transmission varied from a trace to 10%. Symptoms on these hosts were suggestive of infection by mild strains of cucumber mosaic virus. Further, sap-transmission trials to *N. glutinosa*, *Spinacia oleracea*, *Beta vulgaris*, *Cucumis sativus*, *Capsicum annuum*, *Vigna sinensis*, *Chenopodium amaranticolor*, *Cucurbita pepo*, *Datura stramonium*, and *Zinnia elegans* confirmed the difficulty of virus transmission from *Hibbertia*. Yarwood's leaf disc method (4), employing fresh material, also was tested, but was less efficient than the sap-transmission technique. In an attempt to obtain improved recovery of the virus, clones 1 to 6 were placed in a 15.5°C constant temperature chamber in the greenhouse. After 4 weeks, infective sap from the 6 clones growing at 15.5°C was compared with sap taken from a 2nd set of

plants of the same 6 clones growing in the greenhouse. Percentage recovery improved 12-21%.

Because of the low percentage of transmission from *Hibbertia*, a more efficient source plant was needed. Inoculum from *Hibbertia* clones 1 to 6 was compared with similar inoculum taken from each of 6 inoculated *N. glutinosa* plants; comparisons were made on the 13 plant species previously named. Almost 100% transmission was secured on all hosts with inoculum from *N. glutinosa;* with inoculum from *Hibbertia*, transmission varied from a trace to 20%. Virus was easily recovered from most of the test plants and with difficulty from *Spinacia oleracea*, *Capsicum annuum*, and *Beta vulgaris*. Since symptoms on the test plants were identical, irrespective of inoculum source, *N. glutinosa* was used as the source of virus in subsequent sap transmission trials.

Sap transmission from the 8 mosaic-affected *Hibbertia* clones to virus-free plants of *H. volubilis* was done twice. The virus-free plants had been obtained by growing mosaic-affected plants at temperatures above 83°F, and subsequently removing and propagating the new terminal shoot growth which frequently was free of virus. Parallel inoculations to virus-free *Hibbertia* were made with inoculum of isolates 1 to 8 obtained from infected *N. glutinosa*. Symptoms developed 2½-7 weeks after inoculation, and were identical or nearly identical with those manifested by the respective clone, whether inoculum was obtained directly from the clones or indirectly from *N. glutinosa*. Percentage transmission was low, it averaged 12% and varied from a trace to 40%. Recovery inoculations were made to *C. amaranticolor* and to *N. glutinosa*.

Transmission by cutting knife.—Possible transmission of the virus from diseased to healthy plants by cutting knives used in propagation was investigated by passing a scalpel blade through the leaves and stem of a plant showing severe mosaic symptoms. The knife was then used to cut and prepare 10 *Hibbertia* cuttings that were removed from a healthy virus-free plant. Of 110 cuttings, 38 have survived and have been observed for 6 months. To date, the original cutting source plant and all cuttings have remained virus-free.

In a second trial, the knife blade was dipped into a crude virus extract following each cutting. Of 90 cuttings, 23 survived, and only 1 plant developed mosaic symptoms after 6 months. Positive sap transmission to *C. amaranticolor*, *S. oleracea* and *C. sativus* confirmed the presence of the virus in this plant. The healthy plant from which cuttings were taken remained virus-free. The single instance of positive transmission could have resulted from insect transmission. These limited data indicate that the *Hibbertia* mosaic virus is either never or not readily carried on the cutting knife, and that transmission by this means cannot account for the very high prevalence of *Hibbertia* mosaic in commercial and home plantings.

Seed transmission.—Data on seed transmission were not obtained, since a single shipment of 3- and 4-year-old seeds furnished by G. W. Althofer from Australia failed to germinate. As mentioned previously, mosaic-affected *Hibbertia* plants do not produce viable seeds in California. Pollen grains collected in each of 2 years from anthers of different ages were shrunken and shriveled when viewed under the microscope, and did not germinate on water agar in petri plates. This suggests that the failure of *H. volubilis* to produce seeds may be due to formation of nonviable pollen.

Host range.—Because symptoms produced on test plants resembled infection by CMV, isolates 1-8 were compared with 3 strains of CMV on *Beta vulgaris* L. (sugar beet var. Spreckles S.4), *Chenopodium amaranticolor* L., *Spinacia oleracea* L. (spinach var. Prickly Winter), *Datura stramonium* L., *Zinnia elegans* Endl. (zinnia var. Will Rogers), *Cucumis sativus* L. (cucumber var. National Pickling and Ohio MR17), *Cucurbito pepo* L. (pumpkin var. Small Sugar), *Vigna sinensis* (Torner) Savi (cowpea var. Blackeye 5), *Capsicum annuum* L. (pepper var. California Wonder Bell), *Lycopersicon esculentum* Mill. (tomato var. Pearson), *Petunia hybrida* Vilm. (garden petunia select garden mixed), *N. glutinosa* L., and *N. tabacum* L. Two of the CMV strains were obtained from R. G. Grogan, and the third, the Wisconsin 14 strain, from A. O. Paulus.

Symptoms produced on the 13 hosts by the 8 isolates were very similar to (although uniformly less severe than) symptoms produced by the 3 strains of CMV. These symptoms agreed closely with those described for common cucumber mosaic (3). None of the 8 isolates was lethal to any of the 13 host species tested; the isolates were notably mild on spinach, cucumber, pepper, and tomato—host species that are normally killed or severely injured by common strains of CMV. Strains of CMV that are avirulent on tomato are apparently common in England (3). Isolate 2 differed from the other isolates by the yellow mosaic it incited in *N. glutinosa* and spinach. Isolates 5 and 6 were usually slightly more pathogenic on the 13 hosts tested than were the other isolates.

Transmission was secured from 6 additional mosaic-affected *Hibbertia* plants to *S. oleracea*, *C. sativus*, *C. amaranticolor*, and *N. glutinosa*. Four of the 6 isolates produced mild symptoms similar to the 8 isolates described previously; the 2 remaining isolates produced severe cucumber mosaic symptoms on spinach and cucumber. The spinach plants were killed 2-3 weeks after inoculation.

Physical properties of the virus.—Physical property studies of the virus were made with extracts from infected *Hibbertia* and from *N. glutinosa*. The extracts were filtered through cheesecloth and centrifuged at 4,500 rpm for 15 minutes. The supernatant was removed and 15 ml portions were placed in thin-walled Pyrex test tubes. For the thermal inactivation determinations, the tubes were submerged in a circulating water bath maintained at the desired temperature (±0.5°F) for 10-minute periods. Each tube

was cooled rapidly with running water, its contents poured onto a cheesecloth pad, and the pad rubbed over the upper leaf surface of *C. amaranticolor* and *N. glutinosa* test plants. Studies of the longevity of the virus *in vitro* were made by storing the test tubes, prepared as above, at room temperatures (20.5-26.6°C), and at appropriate intervals the content of a single test tube was removed and used to inoculate test plants. In the dilution end point tests, a dilution series was prepared, 1:100-1:1,000,000 and the resulting dilutions inoculated separately on a series of test plants. Physical property characteristics of the 8 isolates, which were compared with those of the Wisconsin 14 strain of CMV and strain CMV1 of Grogan, were identical with respect to the following: 1) dilution end point of 1:100,000; 2) aging *in vitro* of 5 days; and 3) thermal inactivation at 70°C. Physical property characteristics of each isolate were very similar whether virus was recovered from *H. volubilis* or *N. glutinosa*. Smith (3) reports longevity *in vitro* of CMV between 72 and 96 hours. In these tests, a few plants inoculated with 5-day-old sap from each of the 2 CMV strains and each of the 8 *Hibbertia* isolates developed typical symptoms, indicating that CMV is not always inactivated in 3-4 days. Smith (3) reports a thermal inactivation point of CMV between 60° and 70°C. In these tests, virus was inactivated at 70°, but not at 60°C. However, no attempt was made to determine the exact temperature of inactivation.

Cross-protection tests.—Cross-protection tests were conducted with the 8 *Hibbertia* isolates, with the Wisconsin 14 strain of CMV, and with strain CMV1 of Grogan, using cowpea (Blackeye 5) as the test plant. This strain of cowpea develops a systemic mosaic with CMV as well as the usual local lesions. The tests were conducted as follows: Cowpea cotyledons were inoculated with a strain of CMV, and after systemic invasion had occurred, the mosaic-affected leaves were reinoculated with 1 of the *Hibbertia* virus isolates. At the same time, healthy plants of the same age were inoculated with the *Hibbertia* virus. Protection by the first virus was shown if the second virus either failed to produce local lesions, or produced only a few of them, when local lesion production was apparent on the check plants. Results varied somewhat from test to test, protection was sometimes only partial, but definite evidence of strain relationship was obtained between the 2 strains of CMV and the 8 *Hibbertia* isolates. Both CMV strains gave protection against local lesion formation by each of the 8 *Hibbertia* isolates, and each *Hibbertia* isolate gave protection against local lesion formation by CMV1.

Temperature studies.—The effect of constant temperatures on symptom development of mosaic-affected *Hibbertia* clone 3 was studied in controlled chambers at 10°, 15°, 20°, 23.3°, and 26.7° (±0.5°) C at a light intensity of 1,000 ft-c. Plants were cut back to a uniform height of 4 in. in order to produce new growth. Symptoms were very severe on the new growth at 10° and 15°C, severe at 20°, moderate at 23.3°, and mild at 26.7°C. At 10°C, plants developed severe mosaic symptoms and maximum leaf yellowing and chlorosis, severe leaf distortion and dwarfing, a marked rolling of the leaves, pronounced vein swelling, and leaf surface irregularity. Anthocyanin formation was pronounced on the foliage (75% of the leaf area) and the young stems. Plants were stunted very severely in height (75%), and affected shoots were sometimes killed. At 15°C, foliage symptoms were very severe, but stunting (60%) was somewhat less than at 10°C, and affected shoots were not killed. At 20°C, leaf symptoms and distortion were severe; at 23.3°C, leaf symptoms and distortion were moderately severe, but leaf size and plant height (25%) were much less affected. At 26.7° C, leaf symptoms, dwarfing, and plant stunting (10-15%), were mild; and the terminal and lateral shoots that formed on the rapidly growing plants usually remained free of symptoms for 1-2 weeks. Occasionally the terminal and lateral shoots formed at 23.3°C also remained symptomless for a brief period after differentiation. At all other temperatures, symptoms were persistent and completely systemic.

Similar results were obtained when these experiments were repeated using plants of mosaic-affected clones 1 and 3. A few virus-free plants were available at this time, and were included as checks. The best growth of healthy plants occurred between 15° and 23.3° C. Symptoms manifested by clone 1 at the various constant temperatures were similar to those of clone 3, but were considerably less severe and invariably involved fewer leaves.

A method of producing virus-free Hibbertia shoots.—The effect of relatively high temperatures on mosaic-affected *Hibbertia* was studied in order to obtain virus-free shoots, or virus-free plants. In the first trial, mosaic-affected plants of clone 3 cut back to a height of 4 in. were grown in a box 3.5 x 2 x 2 ft, in which heat was supplied by a heating coil, and air temperature was regulated by a thermostat. Polyethylene plastic was used for the top and sides of the box. In November, temperatures in the box on a greenhouse bench varied during the day 29-43°C, and during the night 29.4-31°C (average temperature 31.5°C). Growth was satisfactory and the first and sometimes the second set of developing lateral shoots showed very mild mosaic symptoms. Shoots formed subsequently usually remained free of mosaic symptoms; and cuttings taken from the 3rd- and later-formed lateral shoots 3-4 weeks after the start of the experiment usually proved to be free of virus (86%). All apical shoots were free of virus. Because of the difficulty of transmitting the virus from *Hibbertia* to test plants, recovery tests were not made. The cuttings were rooted under mist and observed for the development of symptoms. If symptoms did not develop after 4 months, plants were considered free of cucumber mosaic virus.

In a second trial, mosaic-affected plants of clone 3 were cut back to a height of 4 in. and grown in large air-temperature chambers (7 x 4 x 7 ft) in the greenhouse. In 1 chamber, day temperatures were 33°C (±1°) for 12 hours, and night temperatures 21°C (average temperature 26°C); in the second chamber, day temperatures were 36.5°C (±1°) for 12 hours, and night temperatures 21°C (average temperature 28.8°C). In 20 days, all lateral and terminal shoots that were formed at 36.5°C day and 21°C night temperatures appeared to be free of virus symptoms, whereas shoots formed at 33°C day and 21°C night temperatures developed mild to moderate symptoms. Thirty days later, the new lateral and terminal shoots were still free of mosaic symptoms, whereas the infected leaves and shoots that were present initially showed only a mild leaf distortion and a slight elevation of the veins. Cuttings of symptomless shoots formed at 36.5° and 21°C varied as to virus content as shown by presence or absence of mosaic on rooted cuttings. Cuttings of the terminal shoot and of the lateral shoot immediately below it usually were free of the virus (93%); cuttings of symptomless lateral shoots farther down the stem varied as to their virus content. Usually the presence or absence of virus in the lateral shoots showed some relationship to the distance of the lateral shoot from the portion of the stem that last showed symptoms. Thus, cuttings of lateral shoots taken from 2-18 in. from the diseased portions of the plant usually contained virus (87%), whereas cuttings 18 or more in. frequently did not (71%). Cuttings taken from the stem apex were always free of virus. The results obtained with mosaic-affected plants grown at 33° and 21°C for 50 days were very different from results obtained at 36.5° and 21°C. Mild to moderate symptoms of *Hibbertia* mosaic were present on all lateral shoots located on the lower half of the plant, whereas lateral shoots arising from the top fourth or half of the stem were symptomless. However, shoot cuttings from the symptomless portion of the plant usually contained virus except for an occasional (6%) cutting from the stem apex that was free of virus. No attempt was made to remove apical shoots at early stages of differentiation.

In a 3rd trial, mosaic-affected plants of clones 1, 2, 5, and 6 were grown in a greenhouse at night temperatures of 25-27.8°C, and day temperatures of 26-43.3°C. Temperatures averaged 28.3°C over the 3-month experimental period. Plants of clone 1 not only showed mild mosaic symptoms, but also less systemic invasion than did plants of clones 2, 5, and 6. Plants of clones 2 and 6 grew the least and showed the greatest area of systemic involvement; plants of clone 5 showed great variation with respect to symptom severity and extent of systemic invasion. Clone 5, as mentioned previously, appears to be infected with a mixture of strains. Shoot cuttings were made from the stem tips of all plants and rooted under mist. Nine of 10 rooted tip cuttings of clone 1 proved to be free of virus, whereas only 3 of the 32 tip cuttings of clones 2, 5, and 6 were free of cucumber mosaic virus. All 3 virus-free cuttings were from plants of clone 5. These plants had manifested exceptional vigor and growth, and incomplete systemic invasion, as evidenced by the absence of symptoms on the upper half of the plants.

Discussion.—Field-grown and nursery-grown *Hibbertia* in California is uniformly affected with mosaic symptoms. These studies indicate that mosaic-affected plants of 3 different symptom types are caused by strains of common cucumber mosaic virus. Since only 14 isolates were considered, and only 8 were investigated in detail, the range in variability of cucumber mosaic virus is is probably greater than that detected in these studies.

Although CMV is readily sap-transmissible, the virus was not readily transmitted in limited trials with a cutting knife. Transmission by this means cannot account for the extremely high incidence of the disease. Virus-infected cuttings taken from infected plants apparently provide the most probable explanation of the widespread occurrence of the disease. Cuttings taken at any time of year probably contain virus, since greenhouse experiments and field observations indicate that the virus is persistent and systemic throughout the year in the temperate coastal climate of California.

Symptom-free terminal shoots were obtained by growing mosaic-affected *Hibbertia* plants at temperatures that averaged near 28.3°C. Since *Hibbertia* grows well at high temperatures, diseased plants grown under a constant high temperature above 30°C probably would yield a high percentage of lateral as well as terminal shoots free of virus. Entire *Hibbertia* plants might be freed of virus, since Kassanis (2) has shown that plants of *D. stramonium* grown at a constant temperature of 30.7°C for 2-3 weeks were freed of CMV.

No previous report has been made of a virus disease affecting *H. volubilis*. That a virus-like disease may occur on *H. volubilis* in Australia is indicated by letters received from L. L. Stubbs, who reported that all *Hibbertia* plants in the Melbourne Botanic Garden and National Herbarium showed virus-like markings, mostly dark green ring patterns on some leaves.

LITERATURE CITED

1. Endo, R. M. 1960. A virus disease of Hibbertia scandens. (Abstr.) Phytopathology 50:634.
2. Kassanis, B. 1954. Heat-therapy of virus-infected plants. Ann. Appl. Biol. 41:470-474.
3. Smith, K. M. 1957. A textbook of plant virus diseases. Little, Brown and Co., Boston, Massachusetts. 652 p.
4. Yarwood, C. E. 1953. Quick virus inoculation by rubbing with fresh leaf discs. Plant Disease Reptr. 37:501-502.

PHYTOPATHOLOGICAL NOTES

EFFECT OF STORAGE TEMPERATURES ON THE VIABILITY OF LYOPHILIZED USTILAGO AVENAE TELIOSPORES

W. T. Kondo

Formerly, Research Assistant, Department of Plant Pathology, Washington State University, now with the Field Research Staff, California Chemical Company, Ortho Division, Richmond, California.

Scientific Paper 2071, Washington Agricultural Experiment Stations, Project 1285. Supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure No. 171.

There is usually a marked decrease in the viability of teliospores of *Ustilago avenae* (Pers.) Rostr. after a year's storage at room temperature. Although Fischer (1) has shown that certain collections of *U. avenae* retain low viability for more than a year, in general, viability is low after a much shorter period. The usual method of maintaining viability is periodic passage of the fungus through its host. This method is time consuming and increases the chance of contamination.

Lyophilization has been utilized in attempts to increase the longevity of *U. avenae* teliospores. In these studies, attention has been focused on temperature as a factor affecting viability during storage.

MATERIALS AND METHODS.—The lyophile procedures of Graham (2), basically designed after that of Raper and Alexander (3), were used with modifications.

Mature teliospores of races 6 and 7 collected from oats grown in the greenhouse were used. The spore collections of each race were separately mixed to obtain a homogeneous mass of each race. A few mg of teliospores were placed in 10-ml Neutraglass ampules and lyophilized dry (without suspending vehicle) under 200μ vacuum at room temperature for 3 hours. Following the freeze-drying process, the ampules were sealed under vacuum with a gas torch and stored at room temperature (20-30°C), 5°, and −15°C for 1 year. The sealed ampules were checked for vacuum 1 and 2 days prior to storage and again before germination counts were made.

After storage, a given ampule was opened, and the lyophilized teliospores were placed on the surface of an agar plate (2.5% Difco Potato-Dextrose Agar). Then 0.1 ml of distilled water was added and the spores were spread by an L-shaped glass rod over the surface of the agar. The plates then were incubated at 20°C for 15 hours prior to germination counts.

TABLE 1. Mean percentage[a] germination of lyophilized *Ustilago avenae* teliospores subjected to different storage conditions

Treatments	% germination after 15 hours at 20°C	
	Race 6	Race 7
1. Fresh spore material	87	84
2. Nonlyophilized spores (stored 1 yr./room temp.)	3	2
3. Lyophilized spores (tested immediately)	77	57
4. Lyophilized spores (stored 1 yr./vacuum)		
a. room temperature	36	5
b. 5°C	81	77
c. −15°C	86	65

[a] 10 observations of 100 spores/field.

RESULTS AND CONCLUSIONS.—The results from a typical evaluation are presented in Table 1. Viability of teliospores immediately after lyophilization (treatment 3, Table 1) was significantly lower ($P<0.05$) than that of fresh teliospores. Surprisingly, however, germination of lyophilized spores after storage for 1 year at 5° and −15°C was significantly better ($P<0.05$) than immediately after lyophilization. The germination of lyophilized spores stored for 1 year at 5° and −15°C (treatments 4b and c) approached that of fresh nonlyophilized spores (treatment 1). Viability of nonlyophilized teliospores stored at room temperature for 1 year (treatment 2) was so low that they were essentially nonviable for pathogenicity studies. Germination of lyophilized teliospores stored at room temperature (treatment 4a) was higher than that of nonlyophilized teliospores stored at similar temperatures but markedly lower than that of lyophilized spores stored at either 5° or −15°C. Race 7 consistently appeared less viable than race 6.

LITERATURE CITED

1. FISCHER, G. W. 1936. The longevity of smut spores in herbarium specimens. Phytopathology 26:1118-1127.
2. GRAHAM, S. O. 1956. Germination responses of Ustilago tritici (Pers.) Rostr. teliospores in relation to lyophilization. Research Studies, State Coll. of Washington 24:307-317.
3. RAPER, K. B., and D. F. ALEXANDER. 1945. Preservation of molds by the lyophile process. Mycologia 37:499-525.

HERITABLE ALBINISM IN SPHACELOTHECA REILIANA

Philip M. Halisky

Assistant Professor of Plant Pathology, University of California, Davis.

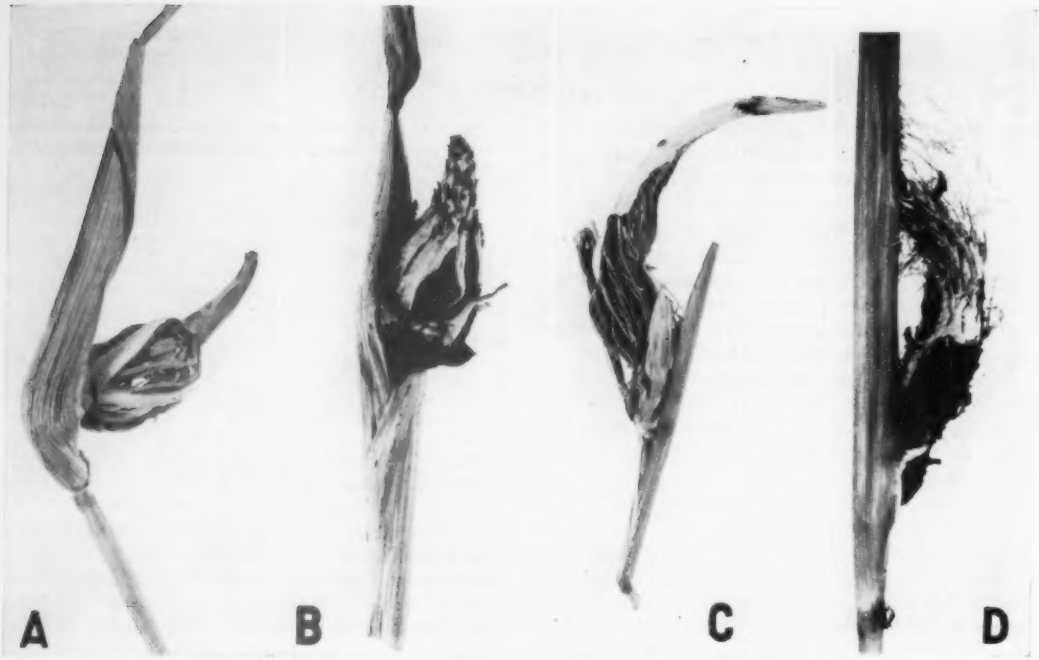


Fig. 1. Two color types of head smut sori produced by *Sphacelotheca reiliana* showing the buff-colored mutant **(A, C)** and common black sori **(B, D)**. The smutted host varieties shown are NK-210 hybrid grain sorghum **(A, B)** and Greenleaf sudan grass **(C, D)**.

Among the most conspicuous examples of mutants in smut fungi are "albino" or "buff" types occurring in species that are commonly dark-spored and characterized by dark sori. These albino or buff mutants produce sori that are conspicuously light-colored as a consequence of hyaline or colorless walls in individual spores. Only a few buff types have been reported, including 1 on wheat, 3 on oats, and 3 on barley, all in the genus *Ustilago* (2). One instance of a mutant albino found outside this genus was reported by Johnson, *et al.* (7) who collected a buff-colored *Sorosporium* on fall panicum. Thus, there are but few reports of albinism in the smut fungi. Fischer and Holton (2) postulated that this rarity may reflect an inherent inability for natural survival in albino smuts, as exemplified by a "white" *Ustilago* with nonviable spores. The scarcity of smut albinos also indicates a probable infrequency of mutations in genetic factors governing spore-wall pigmentation. This report is believed to be the first description of heritable albinism in the genus *Sphacelotheca* and is an extension of information mentioned in a previous abstract (3).

MATERIALS AND METHODS.—During the course of head-smut investigations in California (3, 4, 5), 2 smutted plants of NK-210 hybrid grain sorghum were found with buff-colored sori (Fig. 1). These plants were collected on August 15, 1959, in a large commercial planting of sorghum in Sacramento County during examination of several thousand smut sori. The infection level of head smut in this field was 41% from actual plant counts.

Albinism in smut fungi may be accompanied by such anomalous growth characteristics as inherent nonviability of spores (2) or the production of mycelial rather than sporidial growth upon germination (7). Such aberrant behavior impedes or even prohibits genetic studies in smut mutants. Therefore, the buff-colored sori of *Sphacelotheca* were examined carefully; they contained only hyaline teliospores with smooth walls. Microscopic measurement of 100 such spores indicated an average diameter of 11.2μ. This falls within the range of 9-12μ reported by Fischer (1) for the diameter of typical dark spiny spores of *S. reiliana*. The colorless spores germinated normally on potato-dextrose agar (PDA) producing promycelia and lateral sporidia. The sporidial colonies grew well on PDA through rapid budding and then were transferred to liquid shake cultures containing potato-dextrose broth (PDB). This medium consisted of 10 g of dextrose and the broth from 200 g of boiled potatoes/liter of water. The PDB cultures in 250-ml Erlenmeyer flasks were placed on a rotary shaker for 10 days at room temperature. This method of liquid culture favored sporidial multiplication and was especially suitable for inoculum increase. The turbid PDB cultures containing mass sporidia were mated in desired combinations by mixing the spori-

TABLE 1. Inheritance of homozygous buff-colored sori in 2 selfed generations of *Sphacelotheca reiliana* on 9 sorghums [a]

Host variety	S-1 sporidial inoculum		S-2 sporidial inoculum		% smut [b]
	Stand	Buff smut	Stand	Buff smut	
Ryer-15 sorghum	34	32	33	16	72
Double Dwarf 38 sorghum	92	88	36	29	91
NK-210 hybrid sorghum	49	42	30	15	72
Amak R-10 hybrid sorghum	39	38	33	23	85
Leoti sorgo	26	26	48	27	72
Sumac sorgo	38	30	36	12	57
Sweet sudan	58	34	51	33	62
Greenleaf sudan	84	29	77	54	52
Sudan 23	108	25	64	26	30
Total or avg.	528	344	408	235	62

[a] Data represent number of panicles/stand and total buff-smut sori resulting from inoculating seedlings hypodermically with selfed (S-1 and S-2) smut sporidia.

[b] Infection percentage data are averages of the virulence expressed by both selfed generations of the head smut mutant on each respective host variety.

dial suspensions. The mated lines were then inoculated hypodermically into 2-week-old seedlings of sorghums grown in sterile greenhouse soil. Smutted sori usually emerged about 2 months after inoculation.

PATHOGENIC BEHAVIOR.—Greenhouse studies with 4 grain sorghums, 2 sweet sorgos, and 3 sudan varieties inoculated hypodermically with mass sporidial suspensions indicated that the buff mutant was strongly pathogenic (Table 1). The data show that all 9 varieties tested were substantially smutted, having 30-91% infection (avg. 62%). Spores from the original parent collection (August 15, 1959) germinated normally, producing viable sporidia that were designated S-1 (Table 1). Since the sporidia from individual spores were mated among themselves only, the buff spores were essentially "selfed," hence the designations "S-1" and "S-2." The S-1 sporidial inoculum, injected hypodermically into growing points of seedlings, produced 344 buff-colored sori from a total of 528 sorghum panicles. No black sori were found among the progeny. The teliospores from this smut generation germinated to yield the S-2 sporidial inoculum. Hypodermic inoculation with S-2 sporidial matings produced 235 buff-colored sori in a crop of 408 sorghum panicles. Again, no black sori emerged. The production of 579 buff-colored sori on 9 sorghums by 2 consecutive generations of *S. reiliana* indicates the virulent nature of this mutant. The 579 smut sori were buff-colored, contained hyaline smooth teliospores, and none were typically black (Fig. 1). These facts indicate that albinism in *S. reliana* is a heritable character. The total absence of dark sori among these 579 selfed progeny strongly indicates that the factors governing spore pigmentation, and consequently sorus character, are in a homozygous condition.

In earlier studies, Halisky, *et al.* (3, 5) observed the systemic nature of infection by this smut, often resulting in tattered leaves as a consequence of fungus sporulation in the foliage. Such foliar sporulation again was observed in the present studies on Sweet sudan grass infected with buff smut. Limited greenhouse studies also were conducted with soil-borne teliospores of this mutant. The degree of infection obtained in 4 host varieties was: Leoti 50%; Sumac, 43%; NK-210, 20%; and Greenleaf, 9%. Head smut can be perpetuated by soil-borne teliospores (5).

The existence of a viable pathogenic color mutant in *S. reiliana* is indicative of the variability in this smut fungus. The heritable nature of spore and sorus pigmentation warrants further genetic studies. A complete study of the inheritance of albinism and other spore characters in oat smut fungi was made by Holton (6) and sets a precedent for further studies with the buff mutant in *S. reiliana* described here.

LITERATURE CITED

1. FISCHER, G. W. 1953. Manual of the North American smut fungi. Ronald Press Co. Inc., New York, N. Y. 343 p.
2. FISCHER, G. W., and C. S. HOLTON. 1957. Biology and control of the smut fungi. Ronald Press Co. Inc., New York, N. Y. 622 p.
3. HALISKY, P. M., and L. J. PETERSEN. 1961. Pathogenicity and systemic development of Sphacelotheca reiliana in Sorghum species. (Abstr.) Phytopathology 51:65.
4. HALISKY, P. M., and D. G. SMELTZER. 1961. Head smut disease of corn, sorghum, and sudan grass established in California. California Agr. 15 (1):10-12.
5. HALISKY, P. M., D. G. SMELTZER, and B. R. HOUSTON. 1959. Head smut of sudan grass and sorghum in California. Plant Disease Reptr. 43:1084-1090.
6. HOLTON, C. S. 1936. Inheritance of chlamydospore characteristics in oat smut fungi. J. Agr. Research 52:535-540.
7. JOHNSON, H. W., H. A. RODENHISER, and C. L. LEFEBVRE. 1940. A buff smut of fall Panicum. (Abstr.) Phytopathology 30:12.

COMPARATIVE SURVIVAL OF REHYDRATED AND NONREHYDRATED WHEAT STEM RUST UREDOSPORES ON DRY LEAF SURFACES

Chester R. Leathers

Department of Botany, Arizona State University, Tempe, Assistant Professor, formerly with U. S. Army, Chemical Corps Biological Laboratories, Fort Detrick, Maryland.

Sincere appreciation is given to Dr. K. R. Bromfield for his assistance in the revision of the original manuscript.

Sharp and Smith (3) reported an increase in germinability of dried uredospores induced by exposure to an atmosphere saturated with water vapor. These results have been corroborated in this laboratory and elsewhere. Following the usage of Sharp and Smith, an exposure of spores to a saturated atmosphere prior to use has been rather widely termed "rehydration" or frequently "hydration."

In the course of experiments made in the spring of 1956 to obtain additional information on the behavior of uredospores of *Puccinia graminis* Pers. var. *tritici* (Eriks. & E. Henn.) Shear, Ball, Jacks. & Stakman, the survival on dry wheat leaves of rehydrated and nonrehydrated spores was compared.

Freshly collected uredospores of race 17 produced in the greenhouse on seedling plants of Baart wheat were used for inoculum. A 20-mg portion was rehydrated in a saturated atmosphere for 24 hours and then dusted on the primary leaves of 7-day-old Baart seedlings in a settling tower. A 20-mg portion of nonrehydrated spores was similarly dusted on a set of comparable Baart seedlings. Both sets of plants then were placed in a greenhouse controlled at 75°F and 40% relative humidity. The plants were subirrigated to prevent wetting of the leaf surfaces. At intervals, pots from each set were removed, placed in a dew chamber, and exposed to dew at 75°F for 2 hours to permit spore germination. Germination counts were made directly on leaf surfaces. Only spores whose germ tube length equalled or exceeded the diameter of the spore were considered germinated.

The germinability of rehydrated spores persisted at

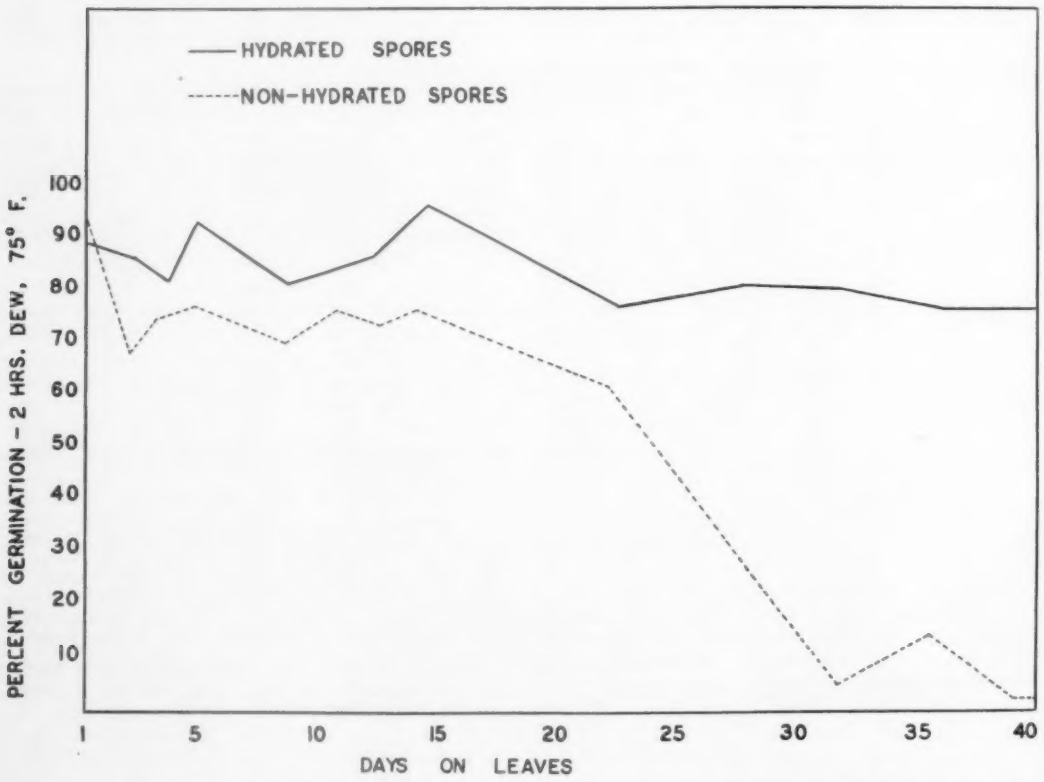


Fig. 1. Germination of uredospores of *Puccinia graminis* var. *tritici*, race 17, held at 75°F and 40% relative humidity on dry foliage of Baart wheat seedlings and then given a 2-hour dew period at 75°F after different intervals.

80-90% for 2.5 weeks. During the next 3 weeks, the germinability was 70-80%. Observations beyond 40 days were impossible because of leaf senescence. The germinability of nonrehydrated spores remained at 70-80% for 2 weeks, then declined rapidly to very low levels during the remainder of the test period (Fig. 1).

In this experiment, rehydrated uredospores retained germinability at a higher level and for a much longer period than did nonrehydrated spores. Wilson (5) reported that inhibitor-depleted uredospores of *Uromyces phaseoli* var. *typica* remained viable longer than did untreated uredospores. L. M. Massey, A. T. Soldo, and R. L. Weintraub (unpublished data) found that the rehydration process may remove an endogenous germination inhibitor present in uredospores of *P. graminis* var. *tritici*. Perhaps, inhibitor removal is a factor in the greater retention of germinability of the rehydrated spores observed in this experiment.

Hwang (1) has estimated that cereal rust uredospores may survive at least 4 days on dry foliage protected from direct insolation. Swaebly (4) reported that uredospores of race 15B survived for 6 days on seedlings of Little Club wheat. More recently, Mohamed (2) presented evidence that uredospores of race 139B survived well for about 7 days on dry leaves of Little Club and Marquis when temperatures averaged 87-90°F in the greenhouse in June and July. Results obtained in the present test indicate that it is possible for nonrehydrated spores to retain relatively high germinability rates in the greenhouse for at least 21 days at a constant temperature of 75°F and a relative humidity of 40%. Rehydrated spores retained high germinability rates for at least 40 days. It is very improbable that spores would survive for such a long period in nature since both temperature and humidity vary greatly under field conditions. If rehydration of spores occurs under natural conditions, however, *i. e.*, if the relative humidity approaches 100% without actual deposition of free water on the leaf surfaces, then perhaps spores can survive for much longer periods than previously suspected.

LITERATURE CITED

1. Hwang, L. 1942. The effect of light and temperature on the viability of uredospores of certain cereal rusts. Phytopathology 32:699-711.
2. Mohamed, H. A. 1960. Survival of stem rust uredospores on dry foliage of wheat. Phytopathology 50:400-401.
3. Sharp, E. L., and F. G. Smith. 1952. Preservation of Puccinia uredospores by lyophilization. Phytopathology 42:263-264.
4. Swaebly, Mary Ann. 1955. Variability in the infection of wheat by Puccinia graminis tritici and studies on extracts from rust uredospores. Unpublished Ph. D. thesis, University of Minnesota, Minneapolis.
5. Wilson, E. M. 1958. Aspartic and glutamic acid as self-inhibitors of uredospore germination. Phytopathology 48:595-600.

VERTICILLIUM WILT RESISTANCE IN PEANUTS (ARACHIS HYPOGAEA)

T. E. Smith

Associate Plant Pathologist, New Mexico Agricultural Experiment Station, University Park.

Journal Series No. 161, Agricultural Experiment Station, New Mexico State University.

In research on pathological and morphological variation of *Verticillium albo-atrum* Reinke & Berth., certain peanut varieties were tested for disease reaction. Greenhouse inoculations were made by dipping the roots of 1-week-old peanut seedlings in macerated fungal cultures increased on oat grain. Field plantings were made on land contaminated heavily by continuous cotton culture. Wilt symptoms on peanuts were characterized by stunting, leaf yellowing, withering, and defoliation combined with brown discoloration of the stele as reported earlier (2). A rating of leaf symptoms was used to compare varieties with "0" indicating no symptoms and "100" indicating complete withering or defoliation.

In greenhouse tests to study host specificity, 10 peanut varieties were inoculated with a total of 8 cultures isolated from 4 hosts. Hosts from which the cultures were originally isolated and their average ratings for disease severity on peanuts were: Cotton, 32, 48, 58, and 66; peanuts, 73 and 46; chile pepper, 57; and *Solanum elaeagnifolium* Cav., 53, with a least significant difference (1%) of 5. The cultures differed in severity of symptoms induced but there was no evidence of exceptional virulence for peanuts in these cultures collected originally from Lubbock, Texas; and eastern and southern New Mexico. Variety averages of disease ratings from this experiment (Table 1) showed significant differences in resistance.

Disease ratings of single plots of several peanut varieties which were grown in an infested field in

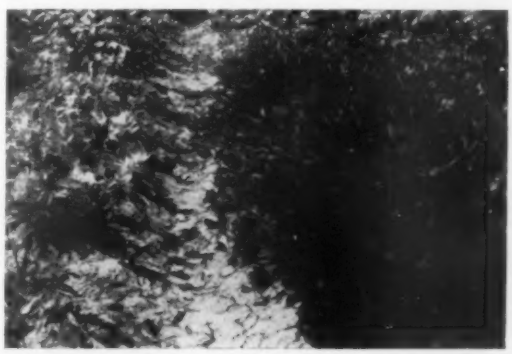


Fig. 1. *Verticillium* resistance in peanuts. Left, a susceptible variety; right, Georgia Bunch 182-28.

TABLE 1. Amount of *Verticillium* wilt on peanut varieties in greenhouse and field, 1960

Variety	Wilt rating [a] in Greenhouse	Wilt rating [a] in Field	Seed Source
New Mexico Valencia	61	84	Commercial variety
Valencia			
PI 119880-2	54	74	R. O. Hammons, through C. H. Hsi, Plains Substation, Clovis, N. M.
PI 152104	69	80	"
PI 210319	63	80	"
PI 210825	69	80	"
Medium Bunch			
Virginia Bunch 67	46	44	W. K. Bailey, Plant Industry Station, Beltsville, Md. through C. H. Hsi
Georgia Bunch 170-11		86	"
Georgia Bunch 177-19	51	68	"
Georgia Bunch 182-28	24	12	"
Georgia Bunch 182-31		80	"
Georgia Bunch 186-28	51	48	"
Spanish			
Small	54	88	W. G. Vinzant, County Agent, Portales, N. Mex.
Dixie		82	R. O. Hammons, Coastal Plain Expt. Sta., Tifton, Ga.
Spanette		88	"
Other			
N.C. 2		42	W. C. Gregory, N. C. State, Raleigh.
N.C. 4		66	"
X + IN 343		86	"
X + IN 226		80	"
L.S.D. 1%	19	6	

[a] "0" = no symptoms and "100" = completely withered.

1959 showed general agreement with the greenhouse data. In 1960 a larger, replicated planting was grown (Table 1). The Valencia and Spanish types were more susceptible than the Bunch types. New Mexico Valencia was highly susceptible and Georgia Bunch 182-28 (Fig. 1) was highly resistant. Stele examinations of Georgia Bunch 182-28 showed that nearly all plants became infected but discoloration was restricted to the crown and roots. The resistant strain was not promising for local commercial culture in tests conducted by Hsi (1) because of late maturity and other factors.

LITERATURE CITED

1. HSI, C. H. 1960. Comparisons of peanut varieties and strains, Portales, New Mexico, 1958-1959. New Mexico Agr. Expt. Sta. Research Rept. 46.
2. SMITH, T. E. 1960. Occurrence of Verticillium wilt on peanuts. Plant Disease Reptr. 44:435.

RHUBARB ROOT NECROSIS: ITS DISSEMINATION, EFFECT ON YIELD, AND CONTROL BY CHEMICAL SOIL TREATMENTS

H. H. Murakishi

Department of Botany and Plant Pathology, Michigan State University.

Journal Article Number 2753, Michigan Agricultural Experiment Station, East Lansing.

The author thanks Dr. H. S. Potter and Mr. Lane Rushmore of the Extension Service for their cooperation and Mr. Richard Crum for technical assistance.

Supported in part by funds supplied by the Michigan Hothouse Rhubarb Growers Association.

Recently a new root necrosis disease (1) of rhubarb (*Rheum rhaponticum* L.) was reported. The disease, caused by a nonsporulating, unidentified fungus, was found in southeastern Michigan where growers have experienced a reduction in hothouse stalk production. Since it appeared that the lower yields might be due to root necrosis, studies were made to determine the mode of dissemination of the root necrosis fungus, its effect on crown size and stalk production, and its response to chemical soil treatments.

Commercially grown hothouse rhubarb is vegetatively propagated by divisions (1 to 2 buds including a portion of the root) which are planted in the field and grown for 2-3 years before crowns reach a size suitable for forcing. To determine the possible mode of dissemination of the root necrosis disease, 2-year-old Victoria crowns and soil samples were obtained from fields where root necrosis occurred. The crowns were washed free of soil; buds were excised and placed into 2 lots: diseased, showing necrotic streaks; and apparently healthy, exhibiting no visible necrosis.

Isolations were made from fresh tissue aseptically removed from the base of the buds after they had been soaked for 30 minutes in 1% formalin. From 121 apparently healthy buds, 9.1% of the isolates on potato-dextrose agar yielded the root necrosis fungus. In the diseased lot, from 191 buds, 24.7% of the cultures yielded the root necrosis fungus. The fungus apparently may be spread in cuttings of plants which are infected, but which show no symptoms.

To determine whether the fungus is soil-borne, healthy Victoria seedlings were grown in 6-in. pots of soil obtained from fields where the disease occurred. For control, 10 seedlings were grown in steamed field soil. Eight months after planting, crowns were removed from the pots, washed free of soil, weighed, and examined for disease. All crowns from unsteamed soil were affected with vascular necrosis, while those from steamed soil were healthy and larger. The root necrosis fungus was recovered only from the affected crowns. The average weight/crown from steamed soil was 194.0 g; from unsteamed soil, 49.6 g.

Further studies were made to determine the effect of the root necrosis fungus on crown size and its ultimate effect on hothouse stalk production. Healthy Victoria seedlings were inoculated by dipping the roots in a comminuted mycelial suspension of 3-week-old potato-dextrose broth cultures. Roots of control plants were dipped in sterile broth. Both series were transplanted to steamed soil-sand-peat (3:1:1) mixture in 6-in. pots. After 6 weeks in the greenhouse, the seedlings were removed from the pots and planted in an experimental plot previously in sod at East Lansing. Six months later, 8 randomly selected crowns were dug from each of the uninoculated and inoculated series, washed free of soil, weighed, and examined for root necrosis. The average weight/crown from the uninoculated series was 8.64 kg; from the inoculated, 3.84 kg. All crowns which developed from inoculated plants were necrotic, whereas control crowns were healthy. The crowns then were reset in steamed, soil-sand-peat mixture in 10-in. pots and forced under simulated commercial hothouse conditions (total darkness at 12° C). Stalks were harvested over a 5-week period during February and March, 1960. The average yield of stalks/crown was as follows: uninoculated, 612 g; inoculated, 280 g. Under the test conditions, infection by the fungus reduced yield by more than 50%.

A soil treatment test was started in June, 1957, on a farm where earlier soil tests had shown root necrosis fungus. Dow MC-2 (methyl bromide, 98% plus chloropicrin, 2%) was applied under a polyethylene cover at the rate of 2 lb/100 ft^2. Terrachlor (pentanitrochlorobenzene, 75%) was applied as a wettable powder at the rate of 250 lb/A and then worked into the soil. This was followed by an application of Vapam (sodium methyldithiocarbamate, 31%) in aqueous solution at the rate of 1 qt/100 ft^2. A water seal was applied immediately over the Terrachlor-Vapam treatment. The randomized plots, each 8 × 16 ft, were replicated 4 times. Seven healthy Victoria seedlings were planted 2 ft apart in a single row of each plot 3 weeks after the chemicals were applied. The plots were maintained through 3 growing seasons and the crowns were lifted in November, 1959. Only the 5 center crowns of each plot (total of 20/treatment) were evaluated for root symptoms, crown development, and stalk production.

Examinations of the roots showed that Dow MC-2 was the only treatment that controlled root necrosis. This was reflected in the respective average weight/crown in the treatments: Dow MC-2, 6.9 kg; Terrachlor-Vapam, 3.0 kg; and control, 2.5 kg. The average yield of stalks/crown was as follows: Dow MC-2, 0.91 kg; Terrachlor-Vapam, 0.24 kg; and control, 0.36 kg. Apparently, methyl bromide fumigation, prior to planting, resulted in greater crown size and yield due to control of root necrosis.

LITERATURE CITED

1. Murakishi, H. H. 1960. An apparently new root necrosis disease of rhubarb. Plant Disease Reptr. 44:186–188.

HEAT-INDUCED SUSCEPTIBILITY OF TOBACCO TO TROPAEOLUM MOSAIC VIRUS

Stephen C. Y. Liu and K. M. Silberschmidt

Respectively, Research Plant Pathologist of "Conselho Nacional de Pesquisas"; and Chief, Section of Plant Physiology, Instituto Biologico, São Paulo, Brazil.

A mosaic disease of nasturtium (*Tropaeolum majus* L.) occurring in Brazil, its distribution in the host plant, and behavior *in vitro* have been studied by Silberschmidt (3). He referred to other known plant viruses affecting nasturtium but did not draw definite conclusions regarding relationships. Further work comparing this virus with other known viruses will be reported elsewhere. In the course of this work, we made experiments on the production of local lesions by *Tropaeolum* mosaic virus. The present paper contains some of these results.

Yarwood (4) reported that when bean leaves were immersed in hot water for a few seconds before inoculation, their susceptibility to tobacco mosaic, spotted wilt, peach yellow bud mosaic, and apple mosaic viruses; and to bean rust, bean mildew, and bean anthracnose was increased. The infection quotients (number of local lesions on the heated leaf divided by the number on the control leaf) rose as temperature increased to the sublethal point. He (5) also demonstrated that when bean, cowpeas, or cucumbers were heated for about 20 seconds at 50° C, 6 hours–3 days after inoculation with several types of lesion-producing viruses, the necrotic lesions induced by these viruses were larger and more numerous in treated than in control plants. Yarwood concluded

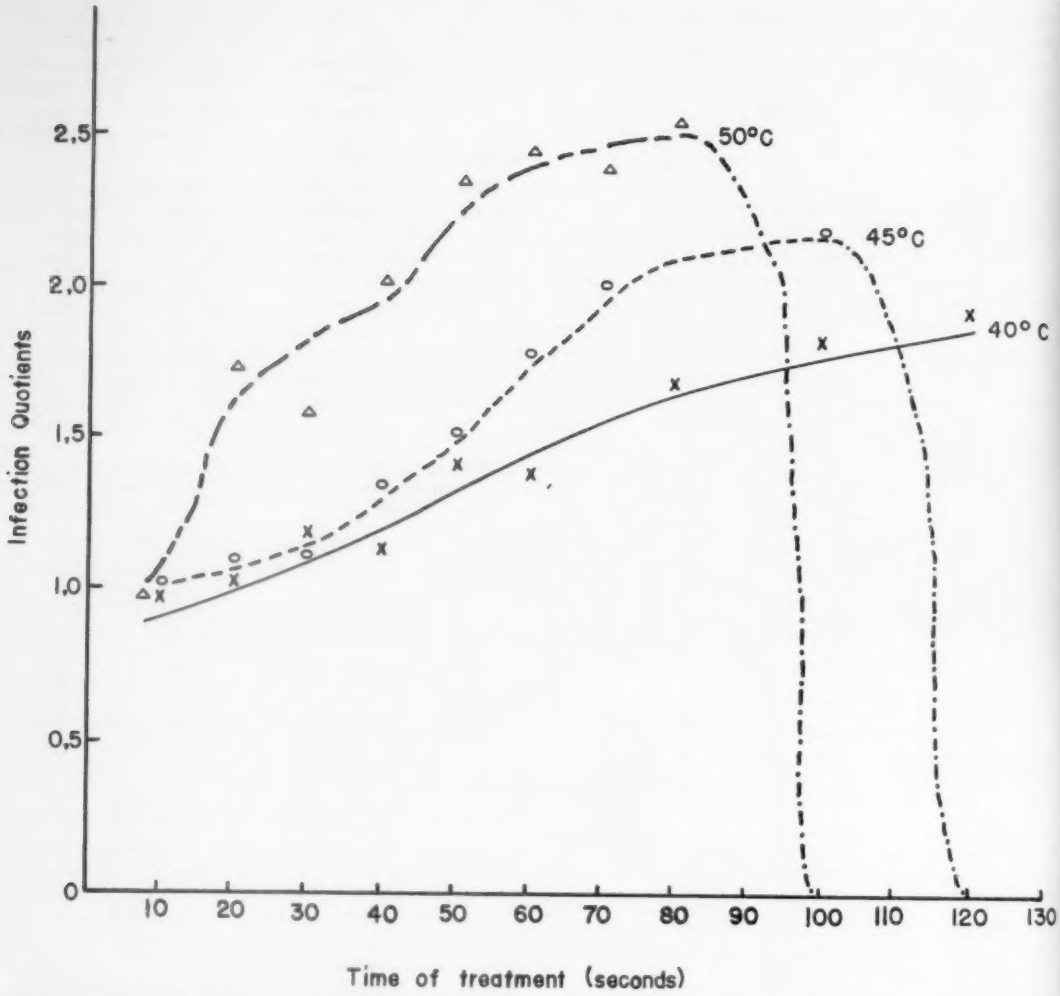


Fig. 1. Infection quotients of White Burley tobacco half-leaves submitted to 40°, 45°, or 50° C for various periods before inoculation with *Tropaeolum* mosaic virus.

that the appearance of more lesions on heated leaves is the result of activation of primary infection which otherwise would remain latent. Resconich (1), too, studied the influence of pre-inoculation treatment of leaves by supra-optimal temperatures on susceptibility of leaves to virus infection, but paid special attention to the effect of heat on starch formation. In bean leaves subjected to this supra-optimal temperature treatment and immediately inoculated with TMV, the heat dosages that increased starch formation were the same as those required for producing maximum susceptibility.

In our studies on nasturtium mosaic virus, we observed that tobacco plants placed in high temperature chambers for a few minutes to several hours before or after inoculation invariably developed more lesions than unheated plants. These results led us to experiments on induced susceptibility of White Burley tobacco to nasturtium mosaic virus.

In each test, all inoculated tobacco leaves were approximately the same size. A method of half-leaf immersion in hot water was developed. The advantage of leaf-halves, as pointed out by Samuel, *et al.* (2), is that they probably have few, if any, differences in susceptibility to virus infection. The use of this technique also makes it possible to obtain significant results from only a few plants. Preliminary experiments showed the method was applicable and satisfactory. Half-leaves treated with hot water (40-50°C) were immediately inoculated, together with nontreated half

Fig. 2. Local lesions on *Nicotiana tabacum* var. White Burley induced by *Tropaeolum* mosaic virus.

leaves. The same quantity of expressed sap was applied to both sides of the leaf and the Carborundum-dusted leaves were inoculated as evenly as possible. After local lesions appeared, usually in 4-8 days, they were counted and infection-quotients calculated. In Fig. 1, the infection quotients, obtained after water treatment in different temperatures, are plotted against the time of treatment. Each point corresponds to the mean value of local lesions produced on a batch of 10 plants.

Tobacco half-leaves, immersed in hot water before inoculation with nasturtium mosaic virus, invariably responded with more local lesions than unheated half-leaves, if there was no injury to the leaves. The optimum length of the heating period, though not determined in detail, depended on the temperature. The infection quotients were higher in plants submitted to higher temperature or in those heated for longer periods. They approached 0 when leaves were injured by heat.

Hot water treatments that induced increased numbers of local lesions generally did not affect lesion size. On both the treated and nontreated half-leaves, lesions were 0.5-2.5 mm in diameter, and appeared similar. Local lesions usually appeared on heated leaf-halves 1-2 days earlier than on nontreated ones. Local lesions were chlorotic to necrotic spots and semicircular to ovoid in shape with a yellowish halo (Fig. 2).

Leaf-halves also were immersed in hot water after inoculation. Such treatments increased the number of lesions too, but the differences were not as striking and dependable as in heat treatment before inoculation.

In our collection of nasturtium virus isolates, we have 1 or 2 variants which, under ordinary conditions, do not produce visible lesions on White Burley tobacco. When test plants were treated with hot water before or after inoculation, however, they developed local lesions after inoculation with these variants. Apparently, certain plant species, in which some viruses are normally latent, may be induced to exhibit local lesions by appropriate heat treatment.

Thus, the results obtained with the half-leaf immersion technique are in agreement with our observation on the effect of high temperatures on local lesion formation by nasturtium mosaic virus. In both cases, treatment increased the number of lesions on inoculated leaves without affecting, in an essential way, the type or size of these lesions.

LITERATURE CITED

1. Resconich, E. C. 1959. Heat-induced susceptibility and thermal injury. (Abstr.) Proc. 9th Intern. Bot. Congr.:324.
2. Samuel, G., R. J. Best, and J. G. Bald. 1935. Further studies on quantitative methods with two plant viruses. Ann. Appl. Biol. 22:508–524.
3. Silberschmidt, K. M. 1953. Studies on a mosaic of nasturtium occurring in Brazil. Phytopathology 43:304–308.
4. Yarwood, C. E. 1956. Heat induced susceptibility of beans to some viruses and fungi. Phytopathology 46:523–525.
5. Yarwood, C. E. 1958. Heat activation of virus infections. Phytopathology 48:39–46.

THE PRESIDENT'S COLUMN

Address of Welcome, Installation of Caribbean Division of The American Phytopathological Society, Miami, Fla., March 21, 1961:

It is with real pride and pleasure that we are gathered here in Miami to celebrate the birth and christening of the youngest daughter of our Society. She has been receiving greetings from our various Divisions. Let me, as the representative from our old parent Society, give you a little family background of our various daughters.

Our eldest daughter is from the Pacific Coast. She is now a mature lady of 47 years. It is not generally known, but the circumstances of her genesis were unusual. In June, 1914, at Davis, Calif., there was created the Western American Phytopathological Society. A year later, this group became the Pacific Division.

This little irregularity long since has been lived down and the Pacific Division has become both famous and powerful. Powerful, we will all agree, judging from the complexion of our Council in recent years. But this dominance probably is only due and fitting for the first born. She also has the biggest grounds, as you might expect of a westerner. They stretch from Arizona to Alaska and on to Hawaii.

The next addition to our family was twins, who arrived in 1918, the Southern and Canadian Divisions. I think you'll agree that they are unlike twins. Our Southern Division, noted for its hospitality, has built up a strong organization and at 43 is in its prime. We expect that our Southern daughter will be sharing some of its room with the new baby. Because of this intimate proximity, she will have more direct contact and will be able to help the baby more than the others.

The second twin is the only daughter that has really left home. She married a Canadian and with her independence is continuing a most active and successful life.

Matters stood this way, with only three daughters for a long time. And then we had a war baby born in 1940, the New England Division. Now, this daughter was also ambitious and she wanted to broaden her horizons. So when she was only 6 years old, she asked for permission to change her status and name. Thus, with the acquisition of New York, New Jersey, and Pennsylvania, she became the present Northeastern Division.

The fifth daughter arrived in 1944 on the banks of the Potomac. She lives on in the old home place. As behooves a resident of our capital city of Washington, she is very discreet, keeps a very trim, if small house, and her advice is much sought.

We now come to our sixth, the North Central Division, born in 1947 and for 14 years, until this very day, our youngest daughter. As you might expect, she is somewhat spoiled and at times wilful. Perhaps the greatest concern to her mother is her resistance to a formal education. She would rather study nature than books and so she will not present formal papers at her meetings. She has a rich farm, however, the bread basket of the United States, the largest membership, and no doubt she will succeed.

So now, newly arrived Caribbean Division, we have described your six elder sisters. You will find a strong family solidarity and, despite individual independence, a keen cooperative spirit and willingness to help, especially in times of stress.

As most of you are aware, in 1958 at Bloomington, Ind., the American Phytopathological Society held its Golden Anniversary with fitting celebrations. Let us go back 53 years to Baltimore, Md., on December 30, 1908. Here were gathered 54 persons, including the founding fathers; C. L. Shear of the U. S. Department of Agriculture; L. R. Jones of Wisconsin; W. A. Orton also of the U.S.D.A.; Donald Reddick and B. M. Duggar of Cornell; A. D. Selby of Ohio; J. B. S. Norton of Maryland; and others. These men had the vision and drive to found a new Society. They said in part, "It is our opinion that an American Phytopathological Society placed on a broad and generous foundation may be of invaluable aid in promoting the future development of this important and rapidly growing subject in America and that its influence may be made of international importance." This was a statement that was to be substantiated many times over in the years ahead. Seven years later, the Society was incorporated under the laws of the District of Columbia. The aims of the new Society were set forth as follows: "To promote investigation and advancement in plant pathology and cooperation among plant pathologists, to hold meetings for the presentation and discussion of the results of research and investigation, to exchange ideas and experiences and consider methods and means of promoting research, to stimulate and encourage the members, to cooperate in all practical ways with other scientific organizations and agencies for the advancement of science and to publish and encourage the publication of contributions to plant pathology." We cannot state our aims more clearly today, nor the aims which should guide our newly created Caribbean Division.

From the beginning, our Society, as a group and as individual members, has been interested in international cooperation, and also specifically in tropical diseases of the Caribbean areas. Our Society was intimately involved in the creation of the Tropical Plant Research Foundation in 1924 to promote research for the advancement of knowledge of the plants and crops of the tropics. One of its major interests was basic research on the control of sugar cane diseases. A founding father, W. A. Orton, was the Scientific Director of the Foundation. Our members have been and continue to be very active in various international, governmental, and foundation agencies to promote, among other things, modern disease control methods. As examples, we might cite the Food and Agricultural Organization of the United Nations, the International Cooperative Administration of the United States, the Rockefeller Foundation, and so on. In particular, we should mention our own Committee on International Cooperation founded some 20 years ago, long headed by that internationalist, Dr. E. C. Stakman, and now in the hands of the first president of the Caribbean Division, Dr. Frederick L. Wellman.

The avowed purpose of the Caribbean Division is to encourage the study and control of plant diseases in the Caribbean area. We can be sure there will be no dearth of problems. Stakman and Harrar in their textbook have described or listed some 60 diseases on the major tropical crops—banana, cacao, citrus, coconut, coffee, sugar cane, rice, and rubber—which they consider merit the rating of diseases of international importance. The programs this morning and afternoon dealt with a score of diseases on some dozen different crops. As we know from experience, even when we learn to control a disease, it cannot be forgotten and there are always new angles to be studied

and improved methods of control to be tried. We can certainly look forward to ever-increasing activity in plant pathology in the Caribbean area.

Is not the creation of the Caribbean Division something even bigger than cooperation in plant pathology? Is it not international cooperation at the grass roots level? Or, perhaps one should say, at the tropical roots level! Most of us would agree that the biggest problem in the world today is a more sympathetic understanding of our neighbor's problems and points of view. The Caribbean Division is an unexcelled opportunity to work this out in practical plant pathology. The eminently successful Canadian Division, while international from the viewpoint of the Society, was of course limited to 1 country outside the United States; and, for the most part, speaking our language. In the Caribbean area, we have a potential of some 15 or more different countries speaking 4 major languages. Cooperation will not always be easy. We know that international cooperation, like true love, does not always run smoothly. At times, the members must exercise great tact, understanding and even fortitude. Perhaps the greatest danger, at least in the beginning, will be a tendency for undue domination by the Division members from the United States. Unless this can be kept successfully in hand, the Caribbean Division will end as it began, merely a beginning. Remember the eyes of the world, at least the plant pathological world, will be upon you as a real practical field experiment in international plant pathology. Let us demonstrate that plant pathologists at least can get on together at the international level. If we can do this, it will lead the way for others to follow.

Now let us return to the seven daughters. Our youngest daughter with her Caribbean background will be warm blooded, a coquette, doubtless difficult at times, but above all, attractive. We can be sure suitors will be in the offing. Just as the Canadian Division grew up, left home, and established her own independent household, we perhaps can expect the same to happen here. If the Caribbean Division can weather successfully the perils of childhood and adolescence, we may in time see the same expression of success. She, too, may leave home and form her own independent Society, perhaps even several societies. Nor, we hope, will she forget her old mother, but will write frequently and come to visit. Like all mothers, however, we hope this is still a long time ahead. In the meantime, we shall look forward to many years in the fold of our Society where we may work intimately together in the solution of our problems in plant pathology. The best of wishes to our Caribbean Division in the years ahead. *Mucha suerte.*

S. E. A. McC.

NEWS

Editor: K. W. KREITLOW, Plant Industry Station, Beltsville, Md.

The Society of American Bacteriologists has changed its name after 61 years of existence. Effective immediately, the Society will be known as the American Society for Microbiology. The new name is considered more descriptive of the broadened scope of the Society's membership and interests. The American Society for Microbiology maintains its headquarters at 19875 Mack Ave., Detroit 36, Michigan.

Plant pathologist Larry L. Krupka, formerly post-doctoral fellow studying host-parasite physiology in the Department of Plant Pathology, University of Nebraska accepted a position with Rohm and Haas Company. Dr. Krupka is now located at Homestead, Florida.

Pathologist Eugene C. Gasiorkiewicz, formerly with Floriculture Crops at the Waltham, Massachusetts Field Station, accepted the position of Plant Pathologist and Horticulturist in the Basic Research Section of S. C. Johnson & Son, Inc. Dr. Gasiorkiewicz is located at the new Agricultural Experiment Station in Racine, Wisconsin.

Plant virologist William Hooker, Michigan State University, visited North Dakota State University during March 20-31 as a participant in the National Defense Fellowship Program for Visiting Lecturers. Dr. Hooker presented lectures on quantitative virus assay using infectivity, serology and physical-chemical methods, and discussed virus multiplication in resistant and susceptible plants.

Pathologist H. H. Thornberry has been granted a sabbatical leave effective March 1 through August 31, 1961. During July, he will travel in western Europe under a grant from AIBS for the specific purpose of organizing an International Center for Technical Information Services in Phytovirology.

Pathologist W. E. Sackston, Professor of Plant Pathology at McGill University, Macdonald College, Quebec, Canada, will be in the U.S.S.R. during May, June and July on a scientific exchange visit in a program sponsored by the National Research Council of Canada and the Academy of Sciences of the U.S.S.R. He will also visit institutions in Lisbon, Madrid, Rome, Copenhagen, Wageningen, Paris, and in the British Isles.

Plant pathologist DeForest H. Palmiter, New York State Agricultural Experiment Station, Poughkeepsie, recently returned from a six months sabbatical leave. While in Chile, Dr. Palmiter made observations in many fruit areas with local agronomists who are interested in disease problems of deciduous fruit crops. He also made visits to the United Fruit Company Research Station in Honduras and the main fruit areas of Argentina where he was invited to check the possible presence of fire blight of apples and pears.

Pathologist Eric G. Sharvelle, Purdue University, presented a paper on "The Fungitoxicity of Guanidine Derivatives" at the International Symposium on Crops Protection held at Ghent, Belgium, May 9. Following the meeting, he spent a month in Europe visiting various commercial and university laboratories and conferring with European biologists on new chemicals for plant disease control.

Plant virologist Lindsay M. Black has been granted sabbatical leave from the University of Illinois for the 1961-62 academic year. Dr. Black plans to spend the year in the laboratory of Dr. Roy Markham, the Agricultural Research Council, Virus Research Unit, Huntington Road, Cambridge, England. He has been given financial assistance through a special fellowship from National Institutes of Health.

Pathologist John W. Oswald, who has served the past two years as Assistant Vice President of the University of California, has been named Vice-President-Executive Assistant. Announcement of the appointment was made by President Clark Kerr following the February 17 meeting of The Regents of the University at Riverside. As Vice-President-Executive Assistant, he will coordinate for President Kerr the activities of the Statewide University's two prinicipal policy-making groups: the President's Council of Chief Campus Officers and the President's Cabinet. He will further serve in the planning and preparations of the President's agenda for Regents' meetings and in the implementation of Regent's action. His responsibilities will also include assistance in the area of administrative planning and the implementation of the University's administrative decentralization and reorganization. Dr. Oswald will retain his appointment as Professor of Plant Pathology and expects to continue to do some teaching and research. He served as vice-chairman of the Department of Plant Pathology at Berkeley from 1954-58.

Representatives of 15 Southern Land Grant Institutes met in Atlanta, Georgia, March 10, with officials of the Southern Regional Education Board to formulate plans for a Regional Graduate Summer Session in Plant Virology. The proposed session would bring together staff members and advanced graduate students for a six weeks period of instruction in plant virology by a faculty of distinguished virologists. Drs. S. J. P. Chilton, J. P. Fulton, H. D. Sisler, and M. K. Corbett, chairman, were named as a committee to work with Dr. William Bowden of the Southern Regional Educational Board to formulate further plans for the proposed summer session in plant virology. Dr. Russell Steere will serve as an advisor to the group. A similar summer session in plant nematology was held at North Carolina State College in 1959.

The Canadian Phytopathological Society will hold its annual meeting at Regina, Saskatchewan, June 26-29, 1961.

The Department of Plant Pathology, University of Arkansas, has recently initiated a study of host-parasite relations using microautoradiographic techniques. These studies are being partially supported by a grant of $22,600 received from the National Science Foundation. Persimmon wilt, crown rust of oats, and cucumber anthracnose are the diseases being investigated. The studies are under the direction of Drs. Charles L. Wilson, Monroe J. Goode, and John P. Jones.

Pathologist C. C. Allison recently returned from the University College of Rhodesia and Nyasaland where he held a Fulbright Lectureship. His assignment in Rhodesia included advising relative to establishment of graduate work and cooperative research projects as well as coordinating research and graduate work. Enroute, Dr. Allison visited several Universities and Research Stations in the Union of South Africa. On his return trip, he visited Universities and Research Institutes in India, Thailand, Japan, and Hawaii as well as the Universities of California and Minnesota.

Pathologist David Gottlieb has been granted a four months' leave of absence to visit laboratories in western Europe, U.S.S.R., India, and Japan where he will confer with research personnel interested in the taxonomy of the Actinomycetes. Dr. Gottlieb is International as well as National chairman of the Committee on Taxonomy of the Actinomycetes. His trip is being sponsored by the National Science Foundation and the American Society for Microbiology.

Congratulations to Dr. D. F. Crossan, Associate Professor, Department of Plant Pathology, University of Delaware, Newark, who received the Christian R. and Mary F. Lindback Award for Distinguished Teaching. This University-wide award carries a citation and a grant of $1000.00.

Professor Emeritus Otto A. Reinking, Cornell University, Ithaca, New York, spent February and March, 1961, as a Tropical Plant Disease Specialist studying decline of coconuts growing in the Territory of Guam. Dr. Reinking was invited by the government of the Territory of Guam, through the U. S. Department of the Interior, to undertake the investigations.